

Capillary Microfluidic Chips for Point-of-Care Testing: from Research Tools to Decentralized Medical Diagnostics

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PAR

Luc GERVAIS

acceptée sur proposition du jury:

Prof. P.-A. Farine, président du jury
Prof. N. de Rooij, Dr E. Delamarche, directeurs de thèse
Prof. J. Brugger, rapporteur
Dr M. Despont, rapporteur
Dr S. Follonier, rapporteur



ÉCOLE POLYTECHNIQUE
FÉDÉRALE DE LAUSANNE

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This thesis is dedicated to my father André, who taught me to be curious and encouraged me to build; to my mother Nancy, who instilled that achievement should be pursued in balance; and to my brothers Patrick, Gabriel and Raphaël, for giving me a thirst for learning while adventuring outside of my comfort zone.

It is not the critic who counts; not the man who points out how the strong man stumbles or where the doer of deeds could have done better. The credit belongs to the man who is actually in the arena, whose face is marred by dust and sweat and blood, who strives valiantly, who errs and comes up short again and again, because there is no effort without error or shortcoming, but who knows the great enthusiasms, the great devotions, who spends himself for a worthy cause; who, at the best, knows, in the end, the triumph of high achievement, and who, at the worst, if he fails, at least he fails while daring greatly, so that his place shall never be with those cold and timid souls who knew neither victory nor defeat. – *Theodore Roosevelt*

Abstract

Research on microfluidic devices for biological analysis has progressed sufficiently to be developed into point-of-care diagnostics products. The goal of this thesis is to improve multiple aspects of capillary-driven microfluidic devices. In particular, the objective is to provide devices with a fast time to result, that are simple to use (one-step), that can be portable, that accept a variety of samples, that operate reliably, that provide a range of detection signals, that are mass manufacturable at low cost, and that are able to detect medically relevant biological molecules.

First, we survey the evolution of microfluidic research into portable medical diagnostic devices. By looking at several gaps and opportunities in current medical diagnostics, we provide an overview of research topics that have the potential to shape the next generation of point-of-care diagnostics. Specifically we explain technologies in the order of sample interacting with different components of a device. We investigate the materials, surface treatments, sample processing, microfluidic elements (such as valves, pumps and mixers), receptors and analytes and the integration of these components into a device that might conceivably leave the laboratory for the hands of consumers.

The knowledge of what is important in a point-of-care diagnostics device was used to develop a proof of concept. One of the main challenges is to make microfluidics easy to use by incorporating reagents and microfluidic elements. We integrated a number of functional elements on a chip such as a sample collector, delay valves, flow resistors, a deposition zone for detection antibodies (dAbs), a reaction chamber sealed with a polydimethylsiloxane (PDMS) substrate, and a capillary pump and vents. We further incorporated capture antibodies (cAbs), detection antibodies (dAbs) and analyte molecules for making one-step immunoassays. The integrated microfluidic chip requires only the addition of sample

to trigger a sequence of events controlled by capillary forces to detect C-reactive protein (CRP), a general inflammation and cardiac marker, at a concentration of 1 ng mL^{-1} within 14 min using only $5 \text{ }\mu\text{L}$ of human serum.

The proof-of-concept is extended to easily modify several assay parameters such as the flow rates and the volumes of samples for tests, and the type of reagents and receptors for analytes. The multiparametric microfluidic chip is capable of analyzing $20 \text{ }\mu\text{L}$ of human serum in 6 parallel flow paths in a range of flow rates with filling times from 10 minutes to 72 minutes. The asymmetric release of dAbs in a stream of human serum is compensated by a Dean flow mixer. Sample is equally split into 6 reaction chambers connected to flow resistances that vary flow rates, and the kinetics of capture of analyte-dAb complexes. The increased incubation time leads to a fourfold increase in detection signal in the reaction chamber with the longer incubation time.

Furthermore, integrating reagents and controlling their release is essential for simple and accurate point-of-care diagnostic devices. We developed reagent integrators (RIs) to release small amounts of dried reagents (ng quantities and less) into microliters of sample. Typical RIs are composed of an inlet splitting into a central reagent channel, with a high hydraulic resistance, and two diluter channels. Reagents spotted in the central channel reconstitute in sample during filling and merge at the end of the RI with a dilution factor corresponding to the relative hydraulic resistance of the channels forming the RI. RIs are simple to integrate in lateral flow assays and provide a great degree of control over reagent integration and dissolution.

Finally, the one-step capillary-driven microfluidic chips have the ability to not only detect a variety of proteins, but also to detect nucleic acids for molecular diagnostics. These devices, especially if manufactured in low cost plastic and used with portable fluorescence readers, have the potential to identify a wide variety of health conditions and to enable truly decentralized medical diagnostics.

Keywords: *point-of-care, POC, diagnostics, fluorescence, immunoassay, protein detection, biosensor, lab-on-a-chip, microfluidics, micro total analysis system, microelectromechanical system.*

Résumé

La recherche sur les dispositifs microfluidiques pour l'analyse biologique a suffisamment progressée pour être transformée en produits de diagnostic au point de soins. L'objectif de cette thèse est d'améliorer multiples aspects des puces microfluidiques à forces capillaires. En particulier, le but est de fournir des dispositifs avec des résultats rapides, qui sont simples à utiliser (une étape), qui peuvent être portables, qui acceptent une variété d'échantillons, qui fonctionnent de manière fiable, qui offrent une gamme de signaux de détection, qui peuvent être fabriqués en masse, et qui sont capables de détecter des molécules biologiques pertinentes au plan médical.

Tout d'abord, nous examinons l'évolution de la recherche en microfluidique vers des appareils de diagnostic médical portables. En regardant plusieurs lacunes et les possibilités actuelles de diagnostic médical, nous donnons un aperçu des sujets de recherche qui ont le pouvoir de façonner la prochaine génération de diagnostics sur le lieu des soins. Plus précisément, nous expliquons les technologies de l'ordre de l'interaction avec l'échantillon des différentes composantes d'un dispositif. Nous élaborons sur les matériaux, traitements de surface, traitement des échantillons, des éléments microfluidiques (tels que valves, pompes et mélangeurs), les récepteurs et les analytes, et l'intégration de ces composantes dans un dispositif qui pourrait éventuellement quitter le laboratoire pour les mains des consommateurs.

La connaissance de ce qui est important dans un dispositif de diagnostic au point de soins a été utilisée pour développer une preuve de concept. Un des principaux défis est de fabriquer des dispositifs microfluidiques facile à utiliser en incorporant les réactifs et des éléments microfluidiques. Nous avons intégré un nombre d'éléments fonctionnels sur une puce, comme un collecteur d'échantillon, les valves de délai, les résistances d'écoulement,

une zone de dépôt pour les anticorps de détection (dAbs), une chambre de réaction scellée par un substrat en polydiméthylsiloxane (PDMS), et une pompe capillaire et des événements. Nous avons également incorporé les anticorps de capture (cAbs), les anticorps de détection et les molécules d'analyte pour faire des immunoessais en une seule étape. La puce microfluidique intégrée nécessite seulement l'ajout de l'échantillon pour déclencher une séquence d'événements contrôlés par les forces capillaires pour détecter la protéine C-réactive (CRP), un marqueur d'inflammation général et cardiaque, à une concentration de 1 ng mL^{-1} , en 14 min et en utilisant seulement $5 \mu\text{L}$ de sérum humain.

La preuve de concept est étendue à fin de pouvoir facilement modifier plusieurs paramètres de dosage tels que les débits et les volumes d'échantillons pour les tests, et le type de réactifs et de récepteurs pour les analytes. La puce microfluidique multiparamétrique est capable d'analyser $20 \mu\text{L}$ de sérum humain en 6 canaux parallèle dans une gamme de débits avec le temps de remplissage de 10 minutes à 72 minutes. Le libération asymétrique de dAbs dans un flux de sérum humain est compensée par un mélangeur de flux Dean. L'échantillon est également réparti en 6 chambres de réaction reliées à des résistances d'écoulement qui varient le débit, et la cinétique de captures des complexes analyte-DAB. Le canal avec le temps d'incubation le plus long conduit à une multiplication par quatre du signal de détection dans la chambre de réaction comparé au canal avec le temps d'incubation le plus court.

En outre, l'intégration de réactifs et le contrôle de leur libération sont essentiels pour des dispositifs de diagnostics au point de soins simples et précis. Nous avons développé des intégrateurs de réactif (RI) pour libérer de petites quantités de réactifs séchés (quantités en ng et moins) dans des microlitres d'échantillon. Les RIs typiques sont composés d'un fractionnement d'entrée dans un canal central pour réactifs, avec une haute résistance hydraulique, et deux canaux de dilution. Les réactifs repérés dans le canal central sont reconstitués dans l'échantillon pendant le remplissage et ils fusionnent à la fin de l'RI avec un facteur de dilution correspondant à la résistance relative hydraulique des canaux formant la RI. Les RIs sont simples à intégrer dans des essais d'écoulement latéral et fournissent un grand niveau de contrôle sur l'intégration de réactif et la dissolution.

Enfin, les puces microfluidiques à forces capillaires ont non seulement la capacité de

détecter une variété de protéines, mais aussi de détecter des acides nucléiques pour le diagnostic moléculaire. Ces dispositifs, en particulier s'ils sont fabriqués en plastique à faible coût et utilisés avec des lecteurs portables de fluorescence, ont le potentiel de détecter un large éventail de conditions de santé et pourraient permettre de véritablement décentraliser les diagnostics médicaux.

Keywords: *diagnostic, fluorescence, immunoessai, détection de protéine, biosenseur, laboratoire sur puce, microfluidique, micro système d'analyse totale, système microélectromécanique.*

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I owe my parents André and Nancy, among a multitude of other things, for giving me the curiosity to combine medicine with technology. I owe my brothers Patrick, Gabriel and Raphaël for training me in the fine scientific art of argumentation. The support and encouragement of friends has been indispensable. For gracefully bearing the ups and downs

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List of Abbreviations

μ TAS	micro total chemical analysis system
BSA	Bovine serum albumin
cAbs	Capture antibodies
CCD	Charge-coupled device
CMOS	Complementary metal-oxide semiconductor transistors
COC	Cyclic olefin copolymer
CRP	C-reactive protein
dAbs	Detection antibodies
dsDNA	Double stranded deoxyribonucleic acid
EDTA	Ethylenediaminetetraacetic acid
ELISA	Enzyme-linked immunosorbent assay
FITC	Fluorescein isothiocyanate
HRP	Horseradish peroxidase
IVD	In vitro diagnostics
kDa	kilo Dalton
MEMS	Microelectromechanical system
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PDMS	Poly(dimethylsiloxane)

PMMA	Poly(methylmethacrylate)
POC	Point-of-care
PSA	Prostate specific antigen
RI _s	Reagent integrators
SA	Streptavidin
SAM	Self-assembled monolayer
SEM	Scanning electron microscopy
ssDNA	Single stranded deoxyribonucleic acid
TRITC	Tetramethylrhodamine isothiocyanate
UV	Ultraviolet light

1

Snapshot of microfluidic chips for point-of-care immunodiagnostics

1.1 Preface

We might be at the turning point where research in microfluidics undertaken in academia and industrial research laboratories, and substantially sponsored by public grants may provide a range of portable and networked diagnostic devices. We provide here a progress report on microfluidic devices that may become the next generation of point-of-care (POC) diagnostics. First, we describe gaps and opportunities in medical diagnostics and how microfluidics can address these gaps using the example of immunodiagnostics. Next, we conceptualize how different technologies are converging into working microfluidic POC diagnostics devices. We explain technologies in the order of sample interacting with components of a device. Specifically, we detail materials, surface treatments, sample processing, microfluidic elements (such as valves, pumps and mixers), receptors and analytes in the perspective of various biosensing concepts. Finally, we discuss the integration of components into accurate and reliable devices. This chapter is based on Microfluidic chips for point-of-care immunodiagnostics, L. Gervais, Nico de Rooij, E. Delamarche, Progress Report, Advanced Materials, 2011.

1.2 Introduction

Large numbers of organic compounds began to be identified and studied to characterize the health of patients in the 19th century. In the second half of the 20th century, tremendous advances in biochemistry and technology (electronics, data processing, mechanics, optics) lead to greater sensitivity and more specific analysis of samples that made clinical chemistry central to medical diagnostics. See for example Durner's review of scientific medicine. [1] Traditionally, selectively chosen analytes were measured during therapy to confirm a tentative diagnosis. Today, a wide variety of measurable analytes, greater sensitivity and a higher informative value of the obtained data increase the usefulness of diagnostics. Diagnostics are performed much earlier to determine predisposition to disease (prevention) or the outcome of disease (monitoring and prognosis). The yearly worldwide *in vitro* diagnostics (IVD) market was \$42 billion in 2007 and is believed to reach \$56 billion in 2012 with 16 companies contributing to 76% of the market. [2]

The research community now has a great opportunity to define the materials, chemicals and analytical techniques that will shape the future of diagnostics. A recent manifestation of this potential is in the emergence of microfluidic bioanalysis. Here, we are interested in understanding where microfluidics can be applied and how they can be developed for specific needs. A first set of questions arises when realizing that in contrast to the large market of \$42 billion for IVD, the yearly worldwide microfluidic market was \$677 million in 2007 [3] with only a fraction ($\sim 47\%$) in the IVD market. At what rate is the microfluidic portion of the IVD market increasing? What gaps in current diagnostics can microfluidics fill? In other words, what benefits can microfluidics provide to the detection of analytes in biological samples? We review some potential answers to these questions by looking at recent progress in the development of materials, functional elements, and their integration into microfluidic devices.

We focus on immunoassays miniaturized using microfluidics. Immunoassays are the most important protein analysis technique. They are a breakthrough analytical technique that made analysis of proteins a medical routine since the pioneering work of Yalow and Berson to determine peptide hormones using radioimmunoassays in the 1950s. [4] Immunoassays have since then improved greatly. The sensitivity limits have been enhanced

to picomolar concentrations using monoclonal antibodies, new labeling techniques and devices for signal transduction and acquisition. It is now possible to routinely determine levels of hormones, cancer markers, response to infection with bacteria and viruses, monitor the evolution of a disease and test for medication levels.

It seems that the best opportunities of microfluidics for diagnostics reside in point-of-care applications because a number of unmet needs can be fulfilled by microfluidics devices due to their portability, short time to result and flexibility. Overall, immunoassays are increasingly ported onto microfluidic formats. [5] Similar approaches to those used in microfluidic immunoassays are shared in diverse applications such as pathogen detection (bacteria, viruses, parasites, etc.) and molecular diagnostics (oligonucleotides) that cannot all be covered in the scope of this progress report. We will for example not cover the state of the art in microfluidics as used for nucleic acid extraction, [6] cell analysis, [7] or blood analysis [8] for which reviews are available.

1.3 Gaps and opportunities in immunodiagnostics

A patient visit to a hospital typically follows these steps: (i) the patient is interviewed by the clinician who follows a differential diagnosis method and considers medical history, risk factors and the current problem, (ii) an examination is performed looking closely at critical aspects from the history and narrowing the list of possible causes, and (iii) tests are performed (such as blood tests and medical imaging) to further narrow the list and/or confirm the differential diagnosis. The investigations are followed by medication, surgery, hospitalization or discharge. Immunodiagnostics are a critical part of the diagnosis process where the identification of key proteins helps to differentiate between major classes of disease: cardiovascular disease, pulmonary disease, infectious disease, metabolic disease (e.g. complications of diabetes mellitus) and cancer (in order of most critical to chronic). The way some diseases are treated has changed with the testing of certain analytes. A high level of cardiac markers (especially CK-MB, myoglobin and troponin I and T) can indicate an increased risk of heart disease [9] and are monitored routinely after a myocardial infarction. [10] Testing for the absence of D-dimer in circulating blood can exclude the presence of a pulmonary embolism. [11] Cancer can be diagnosed by detecting cancer

markers using immunoassays on biopsy samples in conjunction with medical imaging. [12] Cancer markers (such as prostate specific antigen (PSA) for prostate cancer and CA-125 for ovarian and colon cancer) are measured routinely to evaluate the progression of disease and the monitoring of therapy. It is important to note that although many new biomarkers are being discovered, biomarkers must be used within a medical context. The assessment of a clinician in combination with medical imaging and tests for panels of markers lead to a proper diagnosis.

Diagnostics are essential for the identification and treatment of patients for pre-clinical and clinical research. The diagnostic industry is large but it is also very segmented with a few big established companies, many small companies and startups. The trend has been to integrate an increasing number of tests on clinical analyzers. [13] For example, clinical analyzers in the central laboratories of hospitals have evolved to carry out a great range of different tests from many body fluids such as serum and urine (e.g. Roche Cobas® e 602 can perform more than 80 different immunoassays [14]). Devices found in this industry do not mirror consumer electronics where the aesthetics of a device, user friendliness, specifications, portability and interconnectivity are key differentiators that create new and fast changing consumer needs.

There is a pressing need for POC devices as perceived from biohazard threats, the aging population, the spread of infectious disease and the need for home testing and monitoring. The way forward to developing such devices is sensed by looking at grand challenges and long unmet medical needs such as the rapid detection of infectious disease in resource poor settings, [15, 16] early diagnosis of disease via the detection of ultra-low concentration analytes and monitoring of therapy, especially during clinical trials of new drugs.

A striking example of where technology might improve the accuracy challenge of POC diagnostics is illustrated by non-profit organizations that evaluate POC diagnostics for malaria detection, Figure 1.1. [17] There are a variety of malaria POC devices available from different manufacturers all based in this case on an immunoassay test strip similar to a pregnancy test. Manufacturers have the same technology available to them: filter membranes, nitrocellulose membranes, spray deposition of high affinity detection and capture antibodies, labeling of antibodies using colloidal gold or latex particles, detection

protocols and storage methods, yet a huge discrepancy arises in the performance and reproducibility of the different devices. This evaluation of the performance of on the market POC devices illustrates the ubiquitous need for sensitive *and* accurate POC diagnostics, especially for infectious diseases. Ideally, they should also be reliable, low-cost, robust and easy to use. We think that microfluidic technology can lead to more accurate and reliable immunoassays than those performed using test strips owing to (1) a higher degree of control of the flow of liquids in microfluidics, (2) the possibility of miniaturizing test areas and thereby implementing controls and redundant detection regions, and (3) to the improvement of the assay conditions using specific microfluidic elements such as mixers and valves.

1.4 Microfluidics: history and state-of-the-art

Microfluidics technology has a long history from basic research to diagnostic products used in hospitals today, Figure 1.2, see the insightful review by Whitesides. [18] Some of the first research and development projects of manipulating liquids at high precision began with the development of gas chromatography, high-pressure liquid chromatography, [19] and capillary electrophoresis. [20] These techniques revolutionized chemical analysis because they allowed very small samples to be analyzed with high sensitivity.

Meanwhile, the basics of precisely controlling fluids for printing applications began with the development of continuous inkjet technology in the 1950s and the first commercial inkjet printers by Siemens. [21] Inkjet printers were the first microfluidic devices. In the 1970s, IBM licensed continuous inkjet technology and started a large development program for computer printers. Canon and Hewlett Packard independently developed on demand inkjet in the late 1970s and released low cost and mass-market inkjet printers in the 1980s. Hewlett Packard resolved clogging and reliability problem by being first to sell disposable inkjet print heads.

Microfluidics emerged as invaluable tools for analytical applications in chemistry and pharmaceutical research. A high control on materials, interfaces, flow control and dimensionality gave the opportunity to achieve well performing devices. In 1975, Terry developed a silicon micromachined gas chromatograph. [22, 23] A more precise control

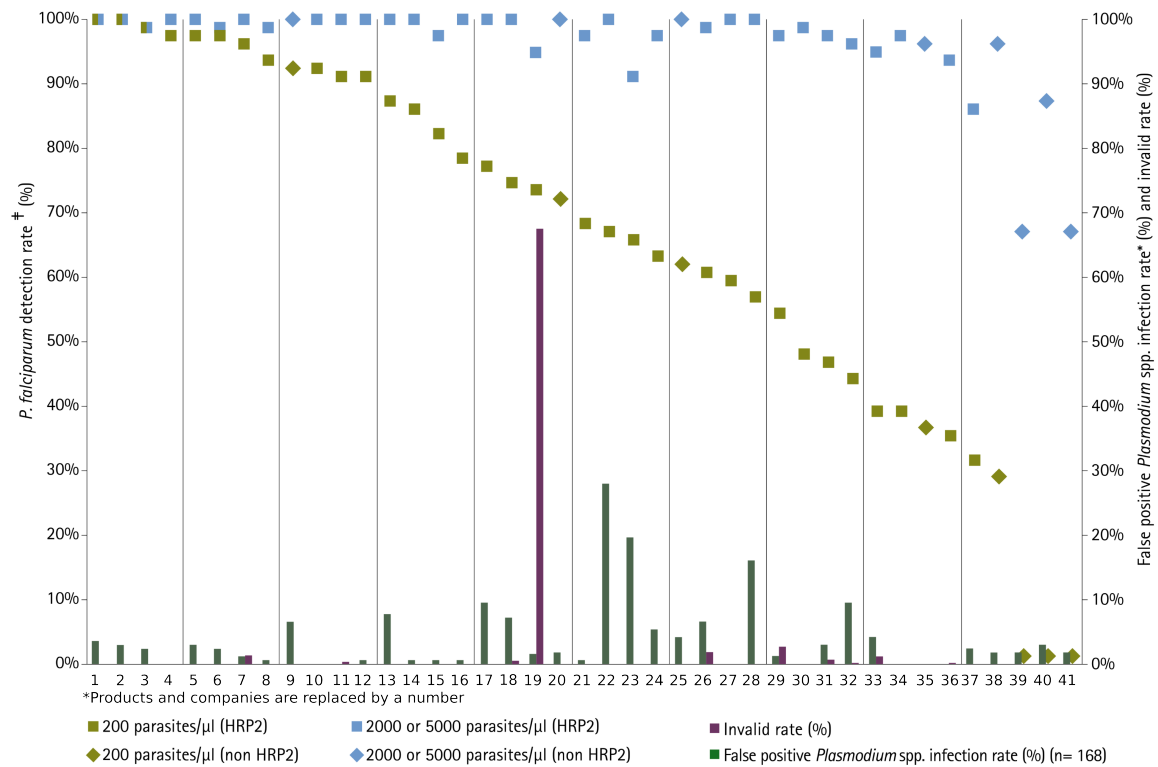


Figure 1.1: Variability in performance of commercially available malaria tests. Blood samples containing *Plasmodium falciparum* at low (200) and high (2000 or 5000) parasite densities (parasites/ μ L) and malaria-negative samples were used to assess the performance of various commercially available malaria diagnostic immunoassay tests. HRP2 stands for histidine-rich protein 2, which is a specific antigen of *P. falciparum*. Tests were plotted in order from best detection rate to worst detection rate. The detection rate is the percentage of *P. falciparum* wild type samples detected by the test. The false positive *P. falciparum* infection rate is the percentage of negative samples identified as positive. The invalid rate is the percentage of tests having a defective control line. There are excellent tests available on the market (on the left) with 100% detection rate in high and low ranges of analyte concentrations, with an invalidity rate below 5% and a false positive rate of 0%. However, the majority of tests do not perform this well, with less than 100% detection rate of high concentrations of analytes and below 75% detection rate of low concentrations of analytes. Alarming, many tests have more than 5% false positive rates and in one case an invalidity rate above 50%. In extreme cases (on the right), tests have a detection rate of less than 70% of high concentrations of analytes and a detection rate of 0% of low concentrations of analytes. Adapted with permission from [17] (where the names of the tests are available), copyright 2008 FIND (Foundation for Innovative New Diagnostics).

of liquid was required for advanced analysis applications leading to the development of micropumps and valves. [24–26] The concept of miniaturized total chemical analysis

systems (μ TAS) was pioneered by Manz *et al.* in 1990 [27] and resulted in the development of microfluidic high-pressure liquid chromatography systems as well as microfluidic capillary electrophoresis in 1992. [28,29]

Following basic research in academia, early microfluidic life science companies appeared such as Caliper Life Sciences, founded in 1995. Cepheid was founded in 1996 and commercialized rapid biothreat detection systems. Hewlett Packard started producing gas chromatography, capillary electrophoresis and high-pressure liquid chromatography systems for laboratories and miniaturizing analytical equipment. Agilent, the measurement and instrumentation division of Hewlett Packard, was spun-off as a separate company in 1999. These companies commercialized the first life science microfluidic products and the first microfluidic capillary electrophoresis systems.

In the 2000s, additional microfluidic diagnostic devices were successfully commercialized. One successful product, the Biosite[®] Triage[®] system [30] provides a convincing implementation of microfluidics as an alternative to nitrocellulose membranes found in pregnancy tests. The chip has a sample metering area, hydrophobic valves to control incubation of the sample with a detection antibody, patterned receptor areas and a microfluidic channel that forms a loop around the main channel, wicking the sample through the device, and keeping the device small. There are more examples of successful microfluidic diagnostic products found on commercial websites and several specialized journals such as *Lab on a Chip*, *Analytical Chemistry* and *Clinical Chemistry*, to name a few.

Well-established diagnostics and life-science companies acquired several of the early microfluidic device companies (e.g. Abbott acquired i-Stat in 2003, Inverness Medical acquired Biosite[®] in 2007, Johnson and Johnson acquired Åmic in 2008). At present, microfluidics cover most, if not all, of the diagnostic segments with devices for pathogen detection, critical care, hematology, and blood typing, for example.

Microfluidics bring the possibility of using small volumes of samples that can be the basis for rapid tests done in a few minutes, that are portable, and provide accurate diagnostics by carefully timing reactions involved in receptor/analyte bindings needed to carry out the test, and by designing accurate signals for a measuring device. Microfluidics

8 Chap. 1 Snapshot of microfluidic chips for point-of-care immunodiagnostics

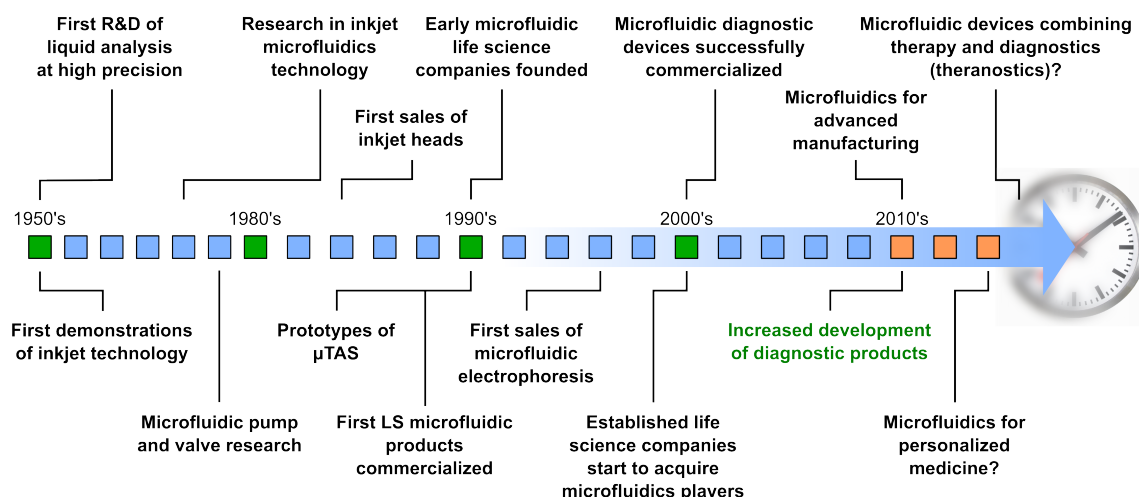


Figure 1.2: Timeline of the evolution of microfluidic technology. Reproduced with permission from IBM, copyright 2010 IBM Corporation.

were initially developed for research in the life sciences and there are now two main applications driving the use of microfluidics in healthcare: POC testing, and central laboratory diagnostics. Microfluidics for research in the life sciences and healthcare submit to different requirements based on the application, Figure 1.3.

Life science research is a large and increasing market for microfluidics. Immunoassays are used for elucidating biochemical pathways, screening for promising drug candidates, studying cell lines and animal models. Due to the large variety of applications, the main selection criterion, flexibility and requirements can vary greatly based on the experiments to be done. Experimentalists often need to change analytes, reagents and the operation of the test. The time to result can vary from seconds to hours. With precious samples, a minute volume might be used and only one analyte measured. The size, weight and instrument cost are not important characteristics. The cost per test can be large or small in the case of multiplexed analysis. Research staff ranges from trained technicians to specialized scientists.

POC testing is beginning to benefit from the huge potential of microfluidics. The degree of integration of a microfluidic technology can vary from having a disposable microfluidic chip used with peripheral equipment (pumps, reader, etc.) to having all functions needed for processing and analyzing a sample and reporting the results on a chip. The main

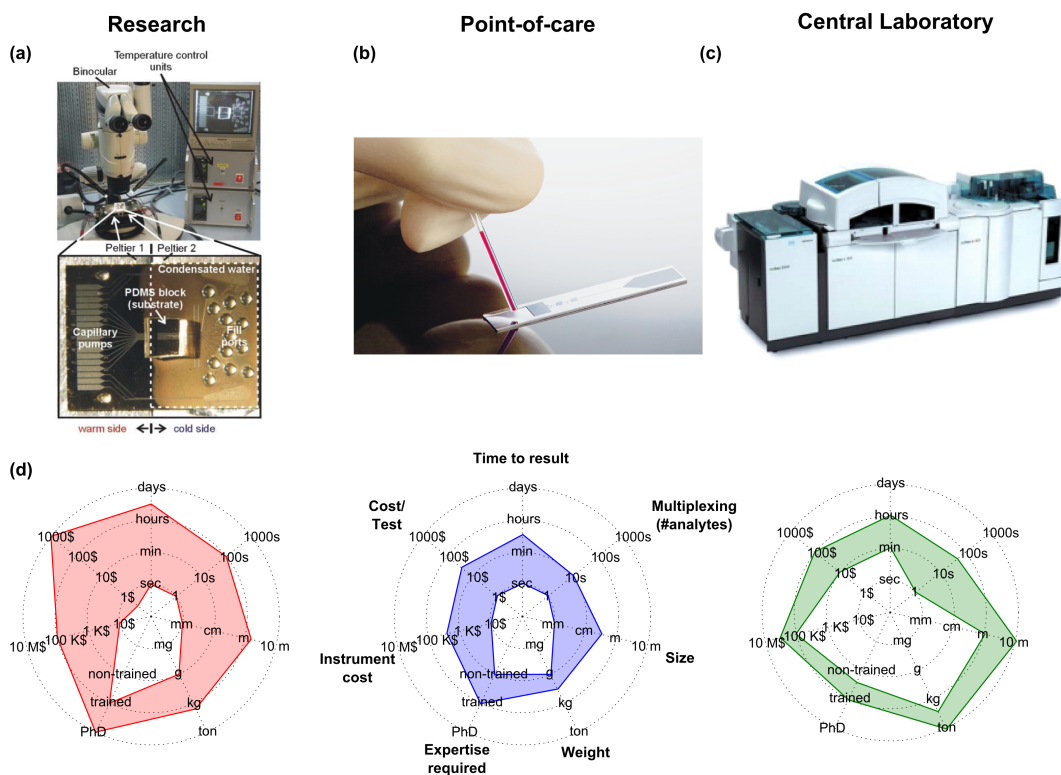


Figure 1.3: Difference in requirements to satisfy an end-user for biological analysis in research, point-of-care testing and central laboratory diagnostics. (a) An example of a research microfluidics platform is shown. It is versatile and can be customized for a wide variety of tests. It requires peripherals such as a microscope, screen, chip, temperature and humidity control. (b) The POC device is small and requires a peripheral reader. [31] It has less ability to multiplex and is not customizable by the user. (c) The central laboratory platform provides over 80 ready to use tests and the system is large. In all three examples, the volume of sample is $\leq 100 \mu\text{L}$ and is not an essential determining factor. (d) Radar charts illustrating the tradeoffs for each biological analysis platform. The units of the different axes shown are common between the charts. Reproduced with permission: (a) from [32], copyright 2004 Royal Society of Chemistry; (b) from IBM, copyright 2010 IBM Corporation; (c) from [14], copyright 2010 Roche; (d) from IBM, copyright 2010 IBM Corporation.

selection criteria are portability, time to result and cost per test. The time to result is between seconds and minutes as devices are often used at the patient side and timely results are required. Multiplexing is usually done for a few analytes. The size and weight of the device are minimal and affect the portability and energy consumption of the reader peripheral. POC devices are used by medical staff. The reader peripheral is not expensive.

The cost per test is low in order for the test to be performed routinely and fit into pricing and reimbursement policies that are relevant for the geography where the tests are performed. As can be seen from above, there is a large number of requirements that POC diagnostics must meet. Technologies for research and central laboratories meet these requirements by using a variety of peripheral equipment, several sampling methods, flexible protocols, and a number of signal detection formats. In contrast, a POC microfluidic device is optimized during manufacturing for a particular application.

Central laboratory testing is done mostly on clinical analyzers. The main selection criteria are throughput and cost per test. Samples are sent from the patient to the central laboratory and placed in a queue with an option for high priority. The time to result can be from several minutes to hours and is usually not critical. Clinical analyzers have a large variety of analysis capabilities and can detect hundreds of analytes. Machines can be meters in size and weigh more than a ton. Operators of clinical analyzers are usually trained technicians. In clinical laboratories that perform a large number of tests, the instrument is often provided and the instrument cost may be small compared to the running costs. The cost per test should be low enough to be done routinely, but can be higher for less common analytes.

1.5 The ideal microfluidic POC device

Now with having outlined the need for POC diagnostics and the background of IVD, we would like to brainstorm on what the ideal microfluidic POC device would be, Figure 1.4. Arguably, the ideal POC diagnostic device would use a small volume of unprocessed sample taken directly from the patient. This volume could be as low as 1 μL , which is significantly less than a drop from a finger prick (typically 25 μL) and is minimally invasive, especially when used with rare samples and patients such as premature babies, newborns, adults suffering from anemia, and the elderly. Accurate sampling would be done by the chip and tests would have the possibility to use an increased volume. The device would multiplex the analysis of up to a 100 analytes that would be a variety of proteins and nucleic acids. Quantitative results would be obtained within 1 minute. The sensitivity limit would be in the picomolar to femtomolar range for detection of analytes

present in low concentrations. The dynamic range of the device would be large, detecting up to micromolar quantities of analytes that have a large concentration range. Analytes would be detected with great selectivity, eliminating cross talk and false positives. Negative controls would also be included. There would be no cross contamination between samples from different patients or different runs. For example, on-going efforts to identify and use biomarkers for the diagnosis, prognosis and treatment of stroke indicate a pressing need for accurate POC tests, which would detect multiple biomarkers with high sensitivity and short turnaround time. [33] Saenger and Christenson review 17 blood biomarkers for stroke. Some of these markers are found at very different concentrations (e.g. lipoprotein-associated phospholipase A2 up to 400 ng/mL, CRP up to a few $\mu\text{g/mL}$, and glial fibrillary acidic protein at a concentration range from ~ 1 pg/mL to hundreds of pg/mL). Some of these markers are predictive, others evolved with different concentration profiles over time after the ischemic attack, and some combination of markers permit differentiating ischemic stroke from other clinical conditions. Arguably, a cheap and easy to use diagnostic device for monitoring some of these biomarkers would be ideal.

The rugged device would be impermeable to water and not damaged when dropped from one meter above ground. It would be made of transparent material where flow should be monitored and optical signal stimulated and read. The device would have a long battery life and could be used for years before recharging. The device would have a shelf life of years when stored between -55°C and 55°C without a reduction in performance. The device would be safe to use in a hospital setting, user-friendly and easy to use by non-technical experts. The device would analyze the sample, calibrate the result, record and transmit encrypted data wirelessly to an electronic health record. To satisfy all actors in the world of diagnostics, except maybe the manufacturer, the disposable part of the device would cost less than one dollar to fabricate. In other words, to be competitive the test would be in the same cost range as the ubiquitous immunoassay strip test.

The ideal POC device imagined above does not exist but crystallizing a vision around it helps identifying desirable characteristics of this device and putting these characteristics in the context of recent progress in the fields of microfluidics and material science. Precise control of materials is essential to realize such a properly working POC device. An

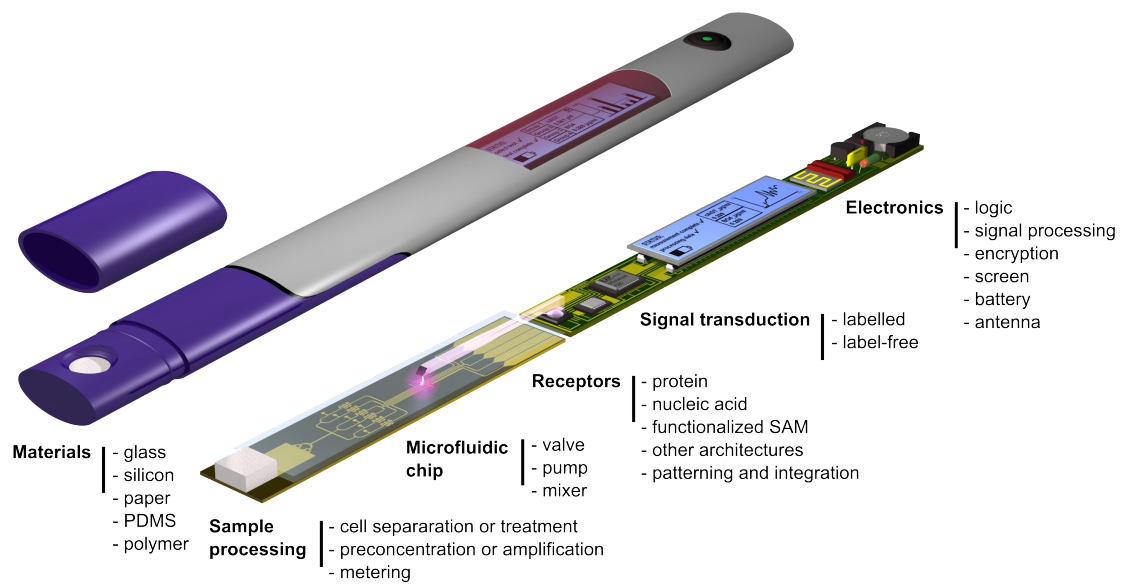


Figure 1.4: The ideal POC diagnostic device. The ideal POC device quantitatively detects several analytes, within minutes, at femtomolar sensitivity from 1 μL of bodily fluid and reports the encrypted results to an electronic health record. The device would have the possibility to use an increased volume of sample with very low concentrations of analytes. The microfluidic chip, shown here encapsulated in purple plastic, is disposable and the mass manufacturing material cost would be less than \$1. This ideal POC device does not exist but research progress in microfluidics and material science point toward the realization of such a POC device in the near future. Reproduced with permission from IBM, copyright 2010 IBM Corporation.

antifouling surface, wettability, filtration/processing of the sample, flow control, advanced signal generation principles, receptor attachment and assembly are all highly dependent on the properties of materials. In this critical review, we focus on discussing recent progress in microfluidic components, biology and hardware. We do not yet describe the information technology part of the device. In the remainder of this chapter, we review the functional elements that are relevant for POC diagnostic devices such as the one discussed here. Elements will appear in the order of interaction with the sample. Finally we will address challenges and opportunities for combining these materials and systems into a device.

1.6 Materials

Materials largely dictate the properties of the microfluidic flow path. The flow rate, capillary pressure, wetting, optical properties, adhesion of biomolecules and the cost of the microfluidic device are all a function of the materials used and their fabrication. Original microfluidic devices were fabricated using the same techniques as microelectromechanical systems (MEMS), *i.e.* using photolithography and etching in a cleanroom. The first generation of microfluidic flow paths were fabricated in silicon and glass. [34] Since then, there has been a veritable explosion of lab on a chip devices fabricated in a wide range of materials, using different fabrication techniques and forming diverse microfluidic systems, Figure 1.5. [35]

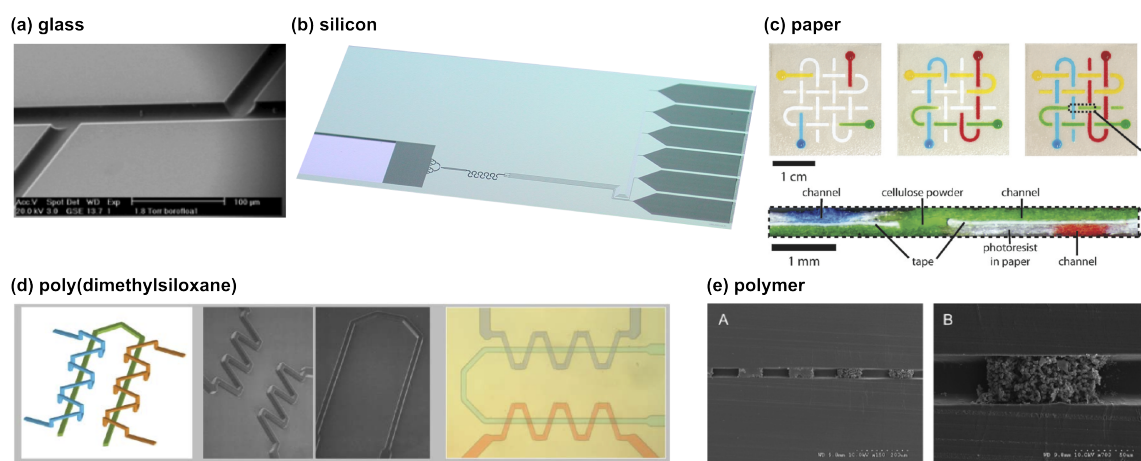


Figure 1.5: Microfluidic flow paths. Microfluidics are fabricated in a variety of materials as illustrated by the examples of (a) a capillary electrophoresis chip in glass, (b) a POC immunoassay chip in silicon, (c) channels in paper, (d) three-dimensional flow paths in PDMS, and (e) micromixers of porous patterned methacrylate in microchannels. Reproduced with permission: (a) from [34], copyright 2003 IEEE; (b) copyright 2010 IBM Corporation; (c) from [36], copyright 2010 American Chemical Society; (d) from [37], copyright 2010 Royal Society of Chemistry; (e) from [38], copyright 2009 Royal Society of Chemistry.

1.6.1 Microfluidic flow paths

We call “microfluidic flow path” the combined geometry and chemistry of the materials used to define the volumes inside which samples flow from an initial

loading zone throughout the microfluidic device. Silicon microfabrication is a reliable prototyping technique for microfluidic devices using existing MEMS and microelectronic infrastructure. However silicon has some disadvantages: it is opaque to ultraviolet and visible light, it is impermeable to gases (this can be a problem when working with cells) and cleanroom fabrication is expensive. It is more practical to make flow paths, valves and pumps in a compliant polymer. Currently in research, a majority of microfluidic flow paths are made in poly(dimethylsiloxane) (PDMS). Whitesides established PDMS as a material of choice for fabricating microfluidic devices. [39] Today, microfluidic channels are commonly etched in silicon and sealed with a PDMS cover in conformal contact. [31]

Increasingly, microfluidic flow paths are often fabricated using polymeric materials, usually using hot embossing for prototyping or injection molding for mass fabrication to produce disposable one-use devices. [40] There are a wide variety of polymers available, which have various optical properties, glass transition temperatures, chemical resistance and permeability to gases and liquids.

Thermoplastic polymers, especially poly(methylmethacrylate) (PMMA) and cyclic olefin copolymer (COC), have emerged as the most used polymers for microfluidic chips. Their properties can vary widely based on the grade of the plastic [41] and they should therefore be chosen appropriately based on the application requirements, such as device operation temperature, transparency and autofluorescence. [42] Disposable diagnostic chips are produced in COC using injection-molding [43] and hot embossing for the fluorescent detection of DNA, [44] with integrated microelectrodes for electrochemistry, [45] magnetic immunoassays, [46] fabricated in PMMA using hot-embossing with integrated waveguides, [47] and with COC integrated waveguides. [48]

Less conventional materials have been used to fabricate microfluidic flow paths. A rapid prototype was fabricated by inkjet printing a flow path of paraffin droplets. [49] Flow paths, valves and pumps have been fabricated in Teflon[®] [50, 51] and fluoroelastomers [52] for use with corrosive substances and solvents. An interesting approach for low cost POC devices is to define microfluidic channels by patterning hydrophobic walls of patterned photoresist or wax on hydrophilic paper or nitrocellulose. [36] An even simpler method is to fabricate flow paths in treated cotton thread with knots for mixing and routing. [53]

1.6.2 Fabrication

Microfluidic fabrication has diverged from conventional fabrication techniques used for microelectronics and MEMS. Microfluidic fabrication uses a variety of materials, the dimensions of chips are larger and key factors are low manufacturing cost and the ability to rapidly prototype new designs. Fabrication approaches to microfluidic chips are being developed and refined such as printing, embossing, molding and nanofabrication techniques [54] and are used to fabricate disposable microfluidic devices. [55]

Photolithography remains an important fabrication step. Multilayer PMMA microfluidic devices were made using a paraffin sacrificial layer. [56] Ultraviolet curable polymers are patterned directly to rapidly prototype microfluidic devices. A number of photocurable polymers have been developed and used to fabricate microfluidic chips such as perfluoropolyether, [57] PDMS containing benzophenone, [58] thiolene-based resin (NOA 81) [59, 60] and polyurethane-methacrylate. [61] Photolithography was used to produce polymer hot embossing masters and stamp thermoplastic polymers chips. [62] Rapid microfluidic chip prototypes are produced with maskless photolithography using a liquid crystal display projector to pattern photoresist [63] and direct lithographic patterning of photoresist using a collimated and focused ultraviolet light emitting diode. [64]

Cleanroom environments for microfabrication are not always available. For this reason, several approaches have been proposed to fabricate microfluidic devices using minimal infrastructure for research prototyping. A laser printer was used to produce masters on copper printed circuit board substrates, followed by etching, and replica molding for the rapid prototyping of PDMS devices. [65] Rapid prototypes of paper based microfluidic devices have been fabricated using ultraviolet light on a sandwich of inkjet printer patterned transparent film, photoresist impregnated paper and black construction paper. [66] A digital craft cutter is used to produce rapid prototypes of flexible microfluidic devices. [67] Microfluidic channels are fabricated quickly by hand by placing a glass fiber between PDMS and a silicon wafer. [68] Microfluidic flow paths are printed onto thermally shrinkable polystyrene sheets [69] and laser ablation is used to micromachine PMMA chips. [70] Arbitrary microchannels can be formed on demand by micropatterned light irradiation of a photoresponsive hydrogel. [71] The strengths of the above-mentioned

methods are clearly their flexibility and their efficiency for easily producing microstructures for microfluidics. However, the accuracy of these techniques is generally in the best case of the order of a few micrometers. Rough surfaces in microchannels may act as pinning sites for moving liquids and may also induce the creeping of liquids along corner/sidewalls, which can lead to the formation of air bubbles.

Unconventional flow path geometries have also been produced for biological experiments. Micromixers of porous polymer have been formed in microchannels. [38] There, the properties of the material used to make the flow path also provide a microfluidic function. Nanochannels with ultra-high aspect ratios of 400 have been fabricated and used to fractionate biomolecules. [72] Microporous thermoformed polymer flexible microfluidic chips have been produced using hot embossing. [73] Tapered channels are produced using diffuse ultraviolet light [74] or laser ablation and integrated into a three-dimensional microfluidic chip. [37] Complex multilevel microchannels are fabricated using three-dimensional photoresist masters. [75] Alternatively, packaging techniques commonly used to fabricate multichip modules (MCM) can readily stack up to one hundred layers of glass ceramic precursors. [76, 77] Each layer is 25 μm in thickness and can be patterned at very high speed by mechanical indentation. After stacking and sintering the layers in a furnace, a microfluidic chip having numerous three-dimensional flow paths is obtained. Modular microfluidic systems have been made with assembly blocks on glass slides [78] and multidimensional systems of assembly blocks on a mainboard. [79] In these last examples, three-dimensional flow paths and modularity induce increased costs and complexity of fabrication.

1.6.3 Surface treatment

Surface treatment techniques for biosensors are well developed and understood. [80] Surfaces in microfluidics play a critical role because they define properties such as wetting, adsorption and repellency of biomolecules, biomolecular recognition using surface-immobilized receptors, sealing and bonding of different materials. Two types of treatments exist to modify the surface properties of microfluidics: wet chemical treatments and gas phase treatments. Wet treatments are simple in terms of infrastructure requirements;

they are flexible and fast to develop from a research standpoint. Surface treatment of microfluidics for production is however best achieved using dry processes based on plasma and chemical vapor deposition. These treatments often eliminate the need for rinsing and drying steps, have high throughput capability and are highly reproducible.

1.6.4 Wet chemical treatment

Among the wet chemical treatments available, the formation of self-assembled monolayers (SAMs) is one of the most versatile and easy to use surface treatments. SAMs have been developed on metals, silicon oxides and polymers. [81,82] Molecules in SAMs pack closely and are composed of a headgroup usually binding covalently to the substrate, an alkyl chain and a terminal functional group. The thickness of the SAM depends on the length of the alkyl chain and density of the molecules on the surface and is typically a few nanometers. SAMs are easy to prepare and can be patterned with sub-micrometer lateral resolution. Different terminal groups can be used for defining the wetting properties of the surface [83] as well as the affinity for or repellency of proteins. [84] For glass surfaces, oxides and polymers that can be oxidized, grafting alkylsiloxanes to surfaces might be the simplest and most economical method. A wettability gradient from superhydrophobic to hydrophilic can be achieved by superposing a SAM-based wetting gradient onto microstructures in silicon that have varying lateral spacing. [85]

Polymeric SAMs can comprise block copolymers and can have various three-dimensional structures, which gives the opportunity to vary their mode of grafting to a surface and the types of functionalities that they carry. Such layers can reach a significant thickness of several hundreds of nanometers and protect/functionalize surfaces more reliably than thinner monolayers. [86] For example, a poly(oligo(ethyleneglycol)methacrylate) polymer brush can coat glass microfluidic chips to make them hydrophilic and antifouling. [87]

Coating polymers onto surfaces to modify their properties is common. For example, poly(ethyleneglycol) is often used to “biologically” passivate microfluidic materials and can be grafted onto PMMA surfaces of capillary electrophoresis microchips to make them hydrophilic. [88] Poly(tetrafluoroethylene) can be used to make chemically resistant

microfluidics devices. [51] Polymeric materials employed to fabricate microfluidics can be modified in many ways. Often, functional groups such as amines or carboxylic acids that are either in the native polymer or added by means of wet chemistry or plasma treatment are used to crosslink proteins and nucleic acids. DNA can be attached to COC and PMMA substrates using surface amine groups. [41] Surfactants such as Pluronic® can be used to make surfaces hydrophilic and protein repellent by adding Pluronic® to PDMS formulations. [89] It is even possible to spin coat a layer of PMMA on a microfluidic chip and “dope” the PMMA with hydroxypropyl cellulose to vary its contact angle. [90]

Proteins themselves can be used on surfaces to change surface wettability, to passivate a surface from non-specific protein binding and for functionalization. Proteins readily adsorb to hydrophobic substrates such as PDMS and polystyrene. By exploiting this property, PDMS substrates can be coated with neutravidin to immobilize biotinylated proteins or biotinylated dextran. [91] Antibody coatings can be optimized depending on the hydrophobicity of the polymeric substrate. [92] Bovine serum albumin is the most commonly used protein to passivate surfaces from non-specific adsorption and is easy to deposit spontaneously from solution to hydrophobic surfaces. On a hydrophilic substrate, a layer of hydrophobic poly(tetrafluoroethylene) can first be coated to enable the subsequent deposition of bovine serum albumin. [93] Heparin, a biological molecule widely used as an anticoagulant, can be deposited from solution onto PDMS to make microchannels hydrophilic while preventing adhesion of blood cells and proteins. [94]

1.6.5 Gas phase treatment

Plasma processing not only can modify the chemistry of a polymeric surface but it also can affect its roughness significantly thereby exacerbating wetting properties to make surfaces superhydrophilic and fluorocarbons can be plasma deposited to make surfaces superhydrophobic. [95] Polymeric surfaces can be patterned using ultraviolet light to initiate radical polymerization followed by covalent grafting of polymers. [96] Plasma-induced grafting is used to attach poly(ethyleneglycol) onto polyamide and polyester surfaces to render them antifouling. [97] Dextran is a polysaccharide comprising of many glucose molecules that can be coated to make hydrophilic antifouling surfaces. A common starting

point to modifying polymers is to introduce surface hydroxyl groups using a plasma treatment followed by grafting a silane and dextran layer. [98] Similarly, PDMS can be superficially oxidized using ultraviolet light for grafting a dextran hydrogel. [99]

The large surface to volume ratio of microfluidic structures makes any potential surface-analyte/reagent interaction a potential issue. Therefore, irrespective of the method used to treat the surfaces of a microfluidic device for POC testing, the surfaces of the device ideally should not attract and deplete analytes or biochemicals that are needed for the test. In addition, surface treatments should not interfere with signal generation and acquisition principles of the device.

1.6.6 Packaging

Microfluidic chips are often composed of multiple layers and components that must be assembled to form the complete device. Devices using PDMS are often sealed reversibly onto substrates by conformal contact mediated by van der Waals forces. Since conformal contact is reversible, it is practical in laboratories where PDMS substrates can be removed and chips reused. Often a stronger adhesion is needed for which a promoter can be used to increase the adhesion of PDMS to silicon, glass and aluminum. [100] In some cases, such as microfluidic devices using high pressures in valves and pumps, surfaces are bonded irreversibly. PMMA and COC substrates are bonded at room temperature using ultraviolet ozone treatment. [101] Various plastic materials are irreversibly bonded with PDMS by hydroxylation of substrates followed by aminosilane treatment of the PDMS, epoxysilane treatment of the plastic, and conformal contact of substrates forming chemical bonds. [102] A straightforward way to permanently bond substrates locally is to use ultraviolet curable adhesive to bond capillary and optical interconnects to chips, [103] glass chips together, [104] and microfluidic stickers on rigid substrates (glass, plastic, metal, etc.). [105] Another alternative is adhesives of complimentary polymeric nanolayers that are deposited using chemical vapor deposition to bond silicon, glass, quartz, PDMS, polystyrene, poly(ethyleneterephthalate), polycarbonate and poly(tetrafluoroethylene). [106]

1.7 Sample processing

A variety of liquid samples from body fluids are routinely analyzed in point-of-care settings, Table 1.1. The volume of sample needed varies according to the concentration of analytes to be detected and to the overall analytical workflow. Samples may need to be pre-processed in order to increase the concentration of analyte and stabilize the sample before analysis.

1.7.1 Body fluids

Common body fluids used for diagnostics are blood, [8] saliva, [107] urine [108] and other fluids sampled directly from the organs. Blood contains a massive amount of information about the body. The entire volume of blood, about 4 L for an average adult, is recirculated in the body every minute. The hematocrit composed of erythrocytes (red blood cells), reticulocytes, platelets and leukocytes (white blood cells), represents 45% of blood volume. The plasma containing proteins (albumin, globulins, clotting factors, etc.), hormones, glucose and ions dissolved in water represents the remaining 55% of blood volume. Plasma without blood clotting factors corresponds to serum. Blood between patients varies and there is therefore a need for processing the sample before analysis. [8]

Saliva can be an alternative to blood as a sample for diagnostics. Whole saliva contents are derived from gingival fluid and contain biomarkers that cross over from blood in the vasculature of the salivary glands. Saliva has the advantage of being collected non-invasively making it easier for patients at home or for patients where blood drawing is difficult. Saliva can provide valuable information, however the concentration of certain markers are not always directly correlated with the concentration of these markers in blood. This is due in part to molecules diffusing differently from plasma to saliva: lipophilic molecules diffuse more easily across cell membranes and epithelial barriers than lipophobic molecules. [107] Similarly, neutral molecules diffuse across these barriers more easily than charged ones. To provide an accurate diagnosis, there must be a direct correlation between the concentration of a biomarker in blood and the concentration in saliva. This can be difficult to achieve because salivary gland function varies, is influenced by the collection

method and the degree of stimulation of salivary flow influencing the concentration of biomarkers and the pH of saliva. Additionally, whole saliva contains proteolytic enzymes and enzymes from microorganisms that can break down certain biomarkers. Despite these limitations, several qualitative tests are commercially available to detect viral infection (such as human immunodeficiency virus), past exposure or immunity to a pathogen (such as influenza or tuberculosis), monitoring of hormones, and detection of illicit drug use. [107]

The analysis of urine, termed urinalysis, is widely used in medical diagnosis. Urine is normally acidic (pH 4.5 to 8) and represents a relatively simple sample: it normally contains no (or very little) cells or proteins, a small amount of urobilinogen (coming from a bacterial heme-degradation pathway), ions such as sodium, potassium, magnesium and calcium, and small amounts of creatinine and catecholamines. [108] Additionally, organic molecules such as urea and uric-acid are present in significant concentration. Dipstick tests are frequently used to diagnose metabolic disorders, kidney and liver dysfunctions, and infections such as urinary tract infection. They are also used to detect pregnancy by measuring the concentration of human chorionic gonadotropin (hCG), drugs of abuse and doping substances. In terms of potential sample processing that might be needed for analyzing urine using a microfluidic chip, removing cells and calcium phosphate crystals present in some samples by means of filtration or centrifugation might be needed.

Sample preparation is growing in importance as microfluidics are more routinely used to analyze a variety of real samples. [109] The cell and protein constituents of diagnostic samples can bring particular challenges in microfluidic devices such as cells clogging microfluidic channels and proteins fouling sensor surfaces. An additional challenge is the variability in composition of samples between patients and the variability in composition of sample in the same patient depending on the time the sample is drawn and the drawing method used. Complex samples need to be processed, removed of cells, cleaned and concentrated before they can be analyzed. The processing protocol is a procedure specific to the target analyte and the sample. There is therefore a need for sample processing in microfluidics for which devices have been developed to separate cells from raw patient samples, sort cells and select cells of interest, lyse cells to analyze intracellular content and preconcentrate analytes.

Table 1.1: Variety of samples for medical diagnostics.

Sample	Relevant composition	Typical tests
Blood	45 % hematocrit: erythrocytes, reticulocytes, platelets, leukocytes 55 % plasma	Blood typing, genetic, cardiovascular, metabolism, infectious disease, cancer, drug and hormone monitoring
Plasma	Albumin, globulins, clotting factors, hormones, glucose and ions	Genetic, cardiovascular, metabolism, infectious disease, cancer, drug and hormone monitoring
Saliva	Proteins, nucleic acids, proteolytic enzymes, enzymes from bacteria, bacteria	Mostly qualitative: genetic, cardiovascular, metabolism, infectious disease, cancer, drug and hormone monitoring
Urine	Metabolites, ions Abnormal: protein, blood cells, pathogens	Metabolism, liver, kidney, pregnancy, drug and hormone monitoring
Stool	Bacteria, fiber, cells Abnormal: blood, undigested nutrients	Digestive tract diseases (liver, pancreas, colon), parasites, infectious disease
Amniotic, cerebrospinal, lymphatic fluid	Cells, proteins and nucleic acids Abnormal: pathogens	Genetic mutations, infectious disease, metabolism
Liquid from puncture of lung, pleura, ascites(abdomen) or joint	Cells, proteins and nucleic acids	Cardiovascular, pulmonary, infectious disease, cancer

1.7.2 Cell separation

The simplest way to process complex samples is to remove cells and solid constituents to obtain the liquid containing the analyte. In central laboratories, centrifugation is routinely used to separate plasma from blood cells. Lab-on-a-disk devices can as well separate blood cells and platelets from plasma by means of centrifugation. [110] A low technology approach for POC settings is to use a filter membrane to trap blood cells. A microchannel packed with microbeads filters blood along the flow path. [111] Saliva from raw patient samples is conditioned off-chip to be compatible with microfluidic immunoassays by depletion of debris, cells and high molecular weight glycoproteins using a membrane filter. [112]

Microfluidic implementations can separate blood cells from plasma. Blood cells travel in a higher flow rate microchannel with plasma travelling in bifurcating channels with lower flow rates. [113] The Zweifach-Fung effect is due to differential pressure drops and shear forces on cells. Parameters that affect the cell removal rate are the ratio of flow rates between the main channel and a plasma separation channel and the ratio of diameters of cells to plasma channel. Higher flow rates of 100 $\mu\text{L}/\text{min}$ and higher temperatures of 37°C in the central channel produces a larger cell-depleted zone where plasma is collected in a side channel. [114]

It is often useful to sort specific cell populations from a complex biological sample. To this end, an array of micropillars can separate blood components by hydrodynamic size into different flow streams of white blood cells, red blood cells, platelets and plasma. [115] In a variation of this idea, a virtual array of pillars using negative dielectrophoretic force can separate red and white blood cells from blood. [116] In hydrophoretic filtration, obstacles alternating on the top and bottom of the channel filter large particles and focus small particles able to pass through the gap to separate red blood cells from white blood cell. [117] Blood cells have been separated from plasma using a cross flow filtration channel and sedimentation of red blood cells. [118]

A number of methods can sort particles but it is not yet clear how they could be implemented in a POC device. These methods are promising but they require additional demonstrations with raw patient samples and minimizing the size and complexity of the

instrumentation. For instance, based on recent examples, particles have been steered and sorted using dielectric force, [119] standing surface acoustic waves, [120] spiral microchannels using inertial microfluidics, [121] inertial focusing, [122] focusing in confined flows, [123] and sorting based on the fluorescence of particles. [124,125]

Functionalized microstructures can sort cells of specific interest such as rare cancer cells that are shed by a primary tumor, circulate in the blood and might seed new tumors. Detecting these cells might inform about the response of a tumor to a particular drug. [126] Rare prostate cancer circulating tumor cells from peripheral blood samples can be captured on micropillar arrays functionalized with prostate-specific monoclonal antibody. [127] Rare circulating tumour cells have been isolated from the blood of metastatic lung, prostate, pancreatic, breast and colon cancer patients using functionalized micropillar arrays. [128] Dielectrophoresis can selectively isolate living human leukemia cells from dead ones using their electrical signatures. [129]

1.7.3 Preconcentration of analytes

Detecting biomarkers with extremely low abundance in pico- or femto- molar levels can be challenging for biosensors. In order to increase the sensitivity, biomolecules can first be preconcentrated. In particular, proteins and peptides can be accumulated using electrokinetic trapping [130] or at the intersection of two perpendicular microchannels [131] with a 10^6 accumulation factor. Electrokinetic accumulation can be used to increase the sensitivity of enzyme-linked immunosorbent assay (ELISA) 65 fold. [132] Proteins can be preconcentrated using absorbent particles in alternating microfluidic flows of lipid and aqueous phase. [133] An alternative is to preconcentrate proteins using filtration. For instance, proteins can be preconcentrated using a porous silica membrane between adjacent microchannels that allows buffer ions to traverse but not larger molecules. [134]

For nucleic acid detection, there are powerful methods to increase their concentration in a sample. Zhang and Xing reviewed nucleic acid amplification and analysis. [135] Polymerase chain reaction (PCR) is the most widely used nucleic acid amplification method in clinical and research laboratories. Many lab-on-chip devices implement PCR protocols. [136, 137] In PCR, the denaturation of double stranded DNA promotes the

next DNA synthesis round. PCR demands precise temperature control and thermocycling between 50°C and 95°C that can be challenging to implement in microfluidic systems. To circumvent this challenge, nucleic acid amplification reactions have been developed to operate at reduced or constant temperatures. Loop-mediated isothermal amplification (LAMP) uses four specially designed primers and a DNA polymerase to amplify nucleic acids at a constant temperature of ~60°C. [138] LAMP has been integrated onto microfluidic chips for the analysis of genes [139] and detection of pathogens. [140] In self-sustained sequence replication (3SR) [141] and nucleic acid sequence based amplification (NASBA), [142] a set of transcription and reverse transcription reactions amplify the nucleic acid. NASBA operates at a constant temperature of 42°C and has been implemented in microfluidic chips. [44, 143, 144] There are more examples of nucleic acid amplification protocols such as rolling circle amplification (RCA), [145] single strand displacement amplification (SDA) [146] and helicase-dependent isothermal amplification. [147]

1.7.4 Buffers and additives

Samples are often prepared and pretreated before analysis. The occurrence of a biochemical reaction and the reaction rate are often sensitive to pH. Biological activity is significantly affected by groups of atoms in biological molecules becoming charged depending on pH. Changing the pH of a sample or adding chemical species to form a buffer can be needed for this reason. Buffering a sample is useful for example to preserve native conformation of protein, to prevent aggregation of biomolecules, or to prevent adverse pH shift when samples partly evaporate or are diluted with liquid reagents on a chip. In blood, the equilibrium between carbonic acid (H_2CO_3) and bicarbonate (HCO_3^-) contributes to maintaining the pH close to 7.40. Intracellular fluid is an equilibrium between dihydrogen phosphate ions (H_2PO_4^-) and hydrogen phosphate ions (HPO_4^{2-}). Phosphate buffered saline (PBS) is based on this chemistry and is one of the most common buffers used in biochemistry; it is also frequently used when a sample is spiked with known amounts of analyte of interest for calibrating or developing microfluidic devices.

Good *et al.* selected a variety of buffers based on criteria of ideal solutions for biochemical reactions. [148, 149] Buffer compounds were selected according to criteria

of value in biochemistry: a pK_a between 6 and 8 where most biological reactions occur, maximal water solubility, cell membrane impermeability, minimal salt effects, soluble cation complexes, minimal optical absorbance of visible or ultraviolet light and ease of preparation.

Samples can be prepared with additives to modify their properties. Anticoagulation compounds such as ethylenediaminetetraacetic acid (EDTA) or heparin are added to blood samples to prevent clotting. In some biochemical assays, it is desirable to analyze the content of cells such as intracellular proteins, organelles, chromosomes, RNA, and DNA. To this end, buffers containing chaotropic salts as cell lysing agents can be used, [150] alternatively to lysing cells using heated surfaces, [151] centrifugation, [152] or inductive heating. [153]

1.8 Valves

Valves are the fundamental unit for controlling the movement of liquid in microfluidic channels. Many different valves are used in microfluidic systems. Oh *et al.* wrote an extensive review of historical and new developments in microvalves. [154] Valve actuation can be active or passive. Active valves actuate using an energy input often coming from a peripheral to the device. Passive valves actuate by exploiting energy potential in the device or the sample. A panel of active and passive valves is shown in Figure 1.6.

1.8.1 Active valves

Active valves can switch between an open and closed state, have fast switching times and low leakage. Active valves are often used to repeatedly dose liquid and control liquid at high pressure, Fig. 1.6a-c. An unresolved challenge remains to minimize the complexity of peripherals to operate active valves. So far, this has been a major bottleneck to their use in POC diagnostics. PDMS valves developed by Quake and colleagues are widely used. They are easy to fabricate and can be integrated massively. In these valves, an elastomeric membrane is formed at the intersection of two perpendicular crossing channels. Pressure applied in the control channel presses down the PDMS membrane and closes the flow channel. This approach has been used in pneumatic latches [155] and normally closed

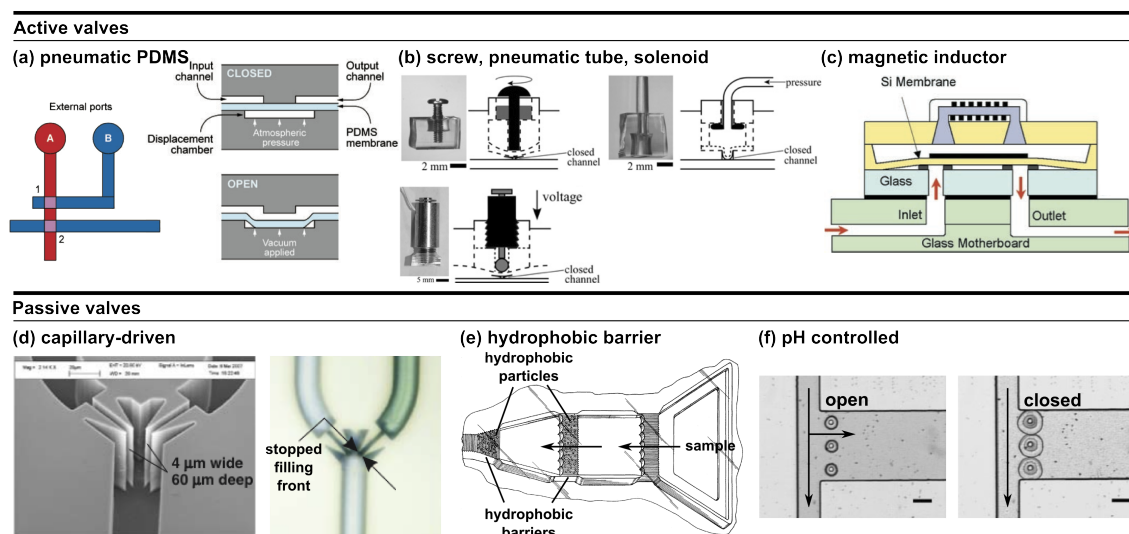


Figure 1.6: Valves. Fluid flow in microfluidic channels can be toggled on and off using valves with active components such as (a) pneumatic latches and valves in PDMS, (b) screws, pneumatic tubes and solenoids, (c) and magnetic inductors; or passive elements using (d) an abrupt change in the curvature of a filling front in a capillary channel, (e) hydrophobic barriers with hydrophobic particles that are gradually made hydrophilic by the sample, and (f) posts of pH sensitive polymers that close a channel in the presence of a solution. Reproduced with permission: (a) from [155] and [156], copyright 2006 Royal Society of Chemistry; (b) from [157], copyright 2009 Royal Society of Chemistry; (c) from [158], copyright 2002 IOP Publishing; (d) from [159], copyright 2008 Springer; (e) from [30], US Patent and Trademark Office; (f) from [160], copyright 2000 Macmillan Publishers.

valves that are opened using vacuum filled control channels. [156] Massively integrated PDMS valves on microfluidic chips have been used for immunoassays [161] and nucleic acid analysis. [162] While these devices are powerful analytical tools in laboratories, they require pneumatic tubes and external pumps making peripheral equipment large and a challenge to use in POC settings.

In order to minimize peripheral equipment, valves based on the opening and closing of PDMS channels have been made using solenoids or manual mechanical actuation. [163] Screws embedded in a layer of polyurethane can be turned to collapse PDMS microchannels and remain in an opened or closed state. [164] Similarly, screws, pneumatic tubes and solenoids can be embedded directly into PDMS microfluidic devices. [157]

Most active valves function by coupling a flexible membrane to an actuator that can be

electrical, magnetic, piezoelectric, thermal or optical. For example a magnetic inductor can produce a force to pull up a silicon membrane electroplated with NiFe permalloy and allowing fluid to flow. [158] Some valves have been made using phase changing materials. For instance, resistive heaters of Pt/Ti can heat PEG and change its phase from solid to liquid with a large change in volume actuating a PDMS membrane microvalve. [165] Shape memory alloy wires of Ni/Ti can be looped around PDMS channels and actuated to squeeze channels closed. [166] The fabrication cost can be minimized even further when there are no microfabricated electrical components on the chip. Valves made of poly(N-isopropylacrylamide) polymer can be actuated using heat from a halogen lamp [167] or a magnetic field heating Fe_3O_4 nanoparticles in the polymer. [168]

1.8.2 Passive valves

Passive valves affect the filling of liquid in the area of the valve but not after this area has been filled. Passive valves function as one directional switches used to delay the flow of liquid or to pause the flow of liquid until the arrival of another. Passive valves are cast into the design and cannot be opened or closed without changing the geometry, Fig. 1.6d-f. This is often sufficient in POC devices carrying out incubation steps and sequential steps. For example, a capillary valve can be made using an abrupt opening in a silicon microchannel. [159] The contact angle of a liquid with a wall is constant as the meniscus moves in a microchannel. An abrupt enlargement of the curvature of the filling front flattens the meniscus and stops the flow of liquid. Trigger valves can be made when a second parallel channel brings liquid to the stopped meniscus and merges with flow continuing in a central channel. Capillary valves are simple to fabricate and integrate but they can be unreliable because they depend on the chemical homogeneity of the surface.

A successful commercial example of a passive valve is the hydrophobic barrier developed by Biosite®. [30] A short section of a capillary channel forms a hydrophobic valve composed of a hydrophobic surface such as plastic or elastomer (polyethylene, polypropylene, polystyrene, polyacrylates and silicon elastomers) and an opposing hydrophilic or hydrophobic surface cover. The hydrophobic surfaces are patterned with hydrophobic particles of latex or hydrophobic polymers of 10 nm and 10 μm in diameter.

Components from the reaction mixture such as proteins, polypeptides, polymers and detergents can bind to the particles. The hydrophobic barrier is changed to a hydrophilic zone by the bound components and the reaction mixture can flow over it. The time for which the hydrophobic barrier can hold depends on the rate of binding and concentration in solution of the components to the hydrophobic barrier. This is particularly useful to allow the optimal amount of time for reagents to incubate with analytes and then to proceed to further processing steps on a chip. Another example of autonomous valves can be made using pH sensitive hydrogel photopolymerized onto micropillars in the middle of a channel. [160] As a solution of pH 11 flows in the side channel, the hydrogel expands blocking the entry of fluid in the channel. Valves fabricated using dots of laser printer toner deposited on a polymer foil can be opened using a semiconductor diode laser. [169]

1.9 Flow control

Accurate flow control in microfluidics is key to the proper functioning of devices. Flow control is provided using a combination of valves, pumps, and resistances. Pumps determine the overall flow rate and volume of liquids used. Laser *et al.* wrote a particularly good review of microfluidic pumps. [170] Flow control of liquids in microfluidics can be achieved using active or passive pumps as is illustrated in Figure 1.7.

1.9.1 Active pumping

In active pumping, the flow rate is controlled using external power sources and can be driven mainly by mechanical displacement, electrical fields, magnetic fields or centripetal force, Fig. 1.7a-d. There are a large variety of microfabricated active pumps mostly using membrane valves moving back and forth. Such pumps can control microliter volumes of liquids in a broad range of flow rates, pressures, viscosities, molecular weights and compositions. However, it is not clear if active pumps could be manufactured for low cost disposable diagnostic devices. Active pumps remain mostly confined to research settings as high-end laboratory devices.

The syringe pump is the simplest microfluidic pump used routinely in microfluidics laboratories. A stepper motor is used to move the plunger in a syringe at a constant

speed defining the flow rate, with capillaries connecting the syringe to a microfluidic chip. The syringe pump can be used for a wide range of volumes and flow rates. Disadvantages are large dead volumes and significant flow hysteresis. Microfabricated mechanical displacement pumps actuate a membrane valve in a reciprocating movement. One such pump uses a piezoelectric actuator compressing and decompressing fluid in the pumping chamber. [171] The membrane valve is placed between a pair of inlet and outlet check valves that direct the flow of liquid. This technology is mature and companies such as Debiotech sell devices. [172] An interesting alternative is to use the mechanical displacement of air bubbles for flow control. An electrolysis-based pump can be fabricated using two air bubble generators activated in sequence to drive a blood sample through a central channel. [173]

Electroosmotic pumping has been known for several decades. It is based on a chemical equilibrium formed at the interface between a liquid and a solid that results in a charged surface and counter ions in the liquid. This is the electric double layer or Debye layer. An applied electric field causes counter ions to move with the electrical current. The ion drag creates a pressure gradient and flow of liquid. In 1997, Ramsey made one of the early demonstrations of a compact format electroosmotic pump. [174] Since then, several highly compact electroosmotic pumps have become commercially available.

Active pumping elements do not necessarily need to be integrated on the disposable device. The device can be kept minimal while having energy provided from a base station. One approach is the Lab on a CD. [175] A liquid is loaded into a reservoir near the center of the disk. The disk is rotated at varying speeds and the liquid is pumped through microfluidic channels towards the outer part of the disk by centrifugal force. Passive capillary valves are used as barriers that liquid can pass when the spinning speed and force is sufficiently high. The speed and flow rate of liquid in a channel between two reservoirs can be calculated as a function of the inner and outer radii (r_0 , r_1), the head of the liquid in the feed reservoir H , the hydraulic diameter (d_H) and length of the channel and the rate of rotation of the disk (ω). Lab on a CD is versatile and it has been used to perform immunoassays from whole blood [110] and the analysis of nucleic acids. [44] A simple peristaltic pump can be fabricated using an elastomeric sheet with a planar microfluidic channel and a stainless

steel ball bearing on top. [176] When a disk containing a rare earth magnet is rotated below, the ball bearing rolls and pumps liquid by compressing the microfluidic channel. Lab on a CD diagnostics and laboratory devices are now available commercially.

Electrowetting devices, often called digital microfluidics, manipulate droplets of reagents and sample on an array of electrodes insulated by a hydrophobic dielectric layer. Applying pulses of electrical potentials between ground and actuation electrodes creates electrostatic and dielectrophoresis forces that temporarily change the wetting properties of the surface from hydrophobic to hydrophilic. Droplets can be moved, split, merged and dispensed from reservoirs. A POC diagnostic immunoassay system has been developed using these principles. [177] The system dispenses blood from a reservoir, mixes the droplet with reagents and performs the optical detection.

1.9.2 Passive pumping

Much like in passive valves, flow rates in passive pumping are encoded in the design. Typical driving forces for propelling liquids in passive microfluidics are chemical gradients on surfaces, osmotic pressure, permeation in PDMS or capillary forces, Fig. 1.7e-f. Such microfluidics fill spontaneously and are appealing owing to their portability, low dead volume and zero power consumption. These devices are clearly attractive because of their low cost advantage. A disadvantage is that flow rates of fabricated devices are set in the design and thus can only be varied to a limited degree.

Porous capillary membranes such as a filter membrane, a nitrocellulose membrane for spotting antibodies and a wicking membrane for pumping are sandwiched together to form an immunoassay strip test such as the Clearblue[®] pregnancy test. [182] An alternative to this approach is to fabricate microchannels in plastic. This provides more reproducible flow rate between devices and volumes based on the dimensions of microchannels. Such devices are manufactured by Biosite[®] [30] and perform a number of POC immunoassays such as for drug testing, cardiovascular disease and infection disease. [179]

Further miniaturized microfluidic channels use smaller volumes of sample and enable a densely packed integration of multiple channels and detection zones in a device. Juncker *et al.* developed autonomous microfluidic capillary systems to perform immunoassays using

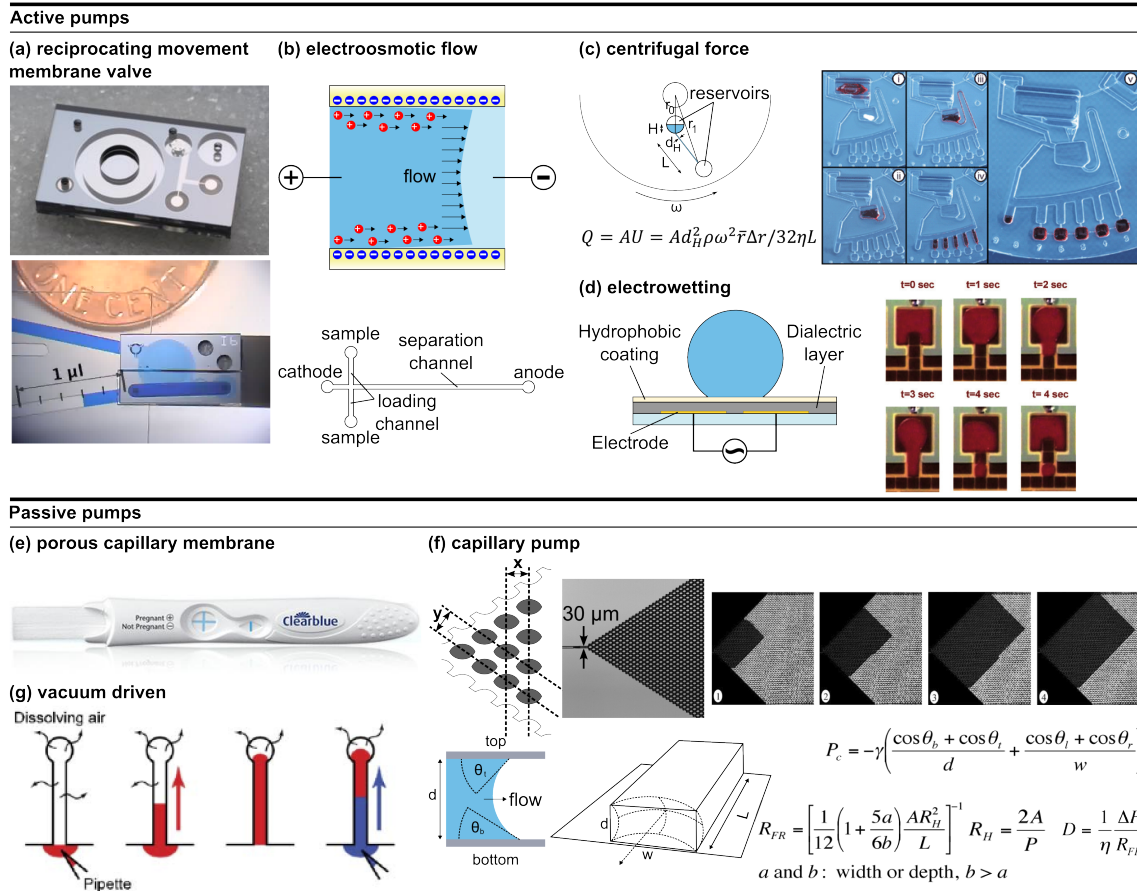


Figure 1.7: Flow control. Active pumps or passive pumps can control the flow of liquid in microchannels. Active pumps function by (a) reciprocating movement of a membrane valve, (b) capillary electrophoresis using electroosmotic flow, [178] (c) centrifugal force in Lab on a CD devices, (d) electrowetting. Passive pumps function using (e) porous capillary membranes such as in the Clearblue[®] pregnancy test, (f) microfabricated capillary pumps, and (g) vacuum driven suction. Reproduced with permission: (a) from [171, 172], copyright 2010 Debiotech; (c) from [175], copyright 1999 American Chemical Society, and from [44], copyright 2010 Royal Society of Chemistry; (d) from [177], copyright 2008 Royal Society of Chemistry; (e) from [179], copyright 2010 SPD Swiss Precision Diagnostic; (f) from [180], copyright 2007 Royal Society of Chemistry; (g) from [181], copyright 2006 Royal Society of Chemistry.

multiple pipetting steps. [183] Capillary forces are used to draw fluid in microchannels with precise control on flow rate and volume. Capillary pressure can be calculated using the depth and width of the microchannels, the surface tension (γ) and contact angles with the bottom, top, left and right side walls. Capillary resistance can be calculated using the dimensions of the microchannel. Flow rate can be calculated using the viscosity (η), the

difference in capillary pressure and the resistance. Capillary pumps have been fabricated using arrays of micropillars. [180] Filling fronts can be precisely controlled and flow rates can be set in the design by changing the dimensions and the wettability of the surface. Wetting can be directionally controlled using regular arrays of asymmetrical micropillars. [184] Flow rates and the total volume used can be tuned by controlling the evaporation rate of liquid in the capillary pump. [185] Capillary microfluidic chips are portable and easy to fabricate but “cast in the design” and do not always scale favorably. For example, when the dimensions of microchannels are large, capillary forces do not dominate and gravity starts to play a role. When dimensions are small, resistance dominates, evaporation in loading pads can be a challenge, and microchannels can block due to defects and dust particles. Nevertheless, if designed properly, capillary driven microfluidic systems have a strong chance of becoming low cost, reliable and state of the art POC devices.

It is even possible to further simplify and/or enhance passive pumping using gravity, vacuum or compressed air. DiagnoSwiss[®] developed a gravity fed immunoassay system that tilts a microfluidic system to control flow rates from 10 to 1000 nL min⁻¹. [186] PDMS degassed in vacuum dissolves air from a microchannel inducing a flow that can be used to perform immunoassays. [181, 187] Pressurized gas can be stored in a chamber that is isolated from a microchannel by a thin membrane. When the membrane is heated and melted, pressurized gas rushes out, pushing liquid into the microchannel. [188] In some cases, a sample can be characterized depending on its flow properties. A plasma sample traveling along a microstructured channel comes into contact with thrombin, inducing clotting and stopping the flow of sample. The distance the sample travels depends on the concentration of fibrinogen. [189]

Interestingly, magnetism can be used to displace a sample in a microfluidic chip by moving only the reagent through a filled channel. This is commonly done using an external magnet and functionalized magnetic particles for sample processing and detection. Magnetism in microfluidics has been reviewed for example by Pamme. [190] In magneto hydrodynamics pumping, a magnetic field is used to move an electrically conducting liquid.

1.9.3 Mixing

Mixing of liquids in microfluidics is often difficult because flows typically are laminar. Turbulent flows are absent and mixing depends primarily on slow diffusion. Several approaches to mixing in microfluidics have been proposed. Active mixers require either external pumps or internal moving parts that are expensive and prone to failure. The best approach is to use passive mixing elements that exploit liquid movement in the geometries of the microchannels to induce mixing. Passive mixers are generally reliable and low cost to fabricate. One example is a staggered herringbone mixer, fabricated with grooves in the floor of a microchannel, producing chaotic flows by inducing a sequence of repeated rotational and extensional local flows. [191] An alternative mixer periodically switches between different Dean vortices in curved microchannels allowing molecules to explore the cross-section of channels chaotically. [192] A mixer using modified Tesla structures divides a flow into two substreams that are merged again and collided in opposite flow directions. [193] Mixing cells of opposite directions are alternated repeating the transverse dispersion from the flow collisions. The simple geometry of these mixers can easily be fabricated and integrated into POC diagnostic devices. A simple mixer can be fabricated by embedding porous patterned methacrylate in microchannels, Fig. 1.7e. [38] The mixer benefits from a short path and efficient mixing at the cost of increased complexity in fabrication and packaging.

1.10 Receptors for analytes

To fabricate an accurate diagnostic device, the appropriate biochemical receptors are chosen to specifically bind to an analyte of interest. By incorporating receptors in advanced architectures, the density of analytes captured can be significantly increased to achieve higher levels of sensitivity in a diagnostic test. An important challenge is patterning or integrating receptors inside microfluidics.

1.10.1 Receptors and architectures

Biological assays use receptors that are specific to ligand analytes, see a few examples in Fig. 1.8a-e. In immunoassays, the ligand is a protein such as an antibody, an immunoglobulin used by the immune system for specific detection of “foreign objects”, *i.e.* antigens, Fig. 1.8a. Monoclonal antibodies are identical immunoglobulins produced by immune cell clones. Polyclonal antibodies are a combination of immunoglobulins produced by many cells during the immune response of an animal to an antigen. An alternative to antibodies, is to isolate only the binding site of an antibody to produce an antibody fragment. [194] Antibody fragments are smaller than whole antibodies and can be packed on a surface to increase the density of captured analytes. Protein scaffolds that are often smaller in size to antibody fragments have been developed. Affibodies are protein scaffolds based on the protein A domain of 58 amino acids. Affibodies capable of specifically binding different analytes can be selected using combinatorial approaches. [195] A number of other protein scaffolds have also been developed and exploited as custom made binding proteins such as synthetic antibodies, [196] ankyrins, anticalins, zinc fingers, and fibronectins. [197]

Oligonucleotides are also used as specific receptors for biochemical recognition, Fig. 1.8b. In a simple case, a surface bound oligonucleotide hybridizes with a complimentary oligonucleotide analyte, and a complementary detection strand. Immobilized oligonucleotides are used to capture complimentary oligonucleotides tagged with capture antibodies. This technique, termed DNA-encoded antibody library, has been used in microfluidic devices for the multiplexed detection of biomarkers from blood. [198,199] Aptamers are oligonucleotides that are selected *in vitro* from libraries of random sequences to bind specifically to molecular targets. [200] In addition to having binding affinities and selectivities rivaling antibodies, aptamers can be chemically synthesized and are more resilient to storage. One way to bind aptamers to a surface is to attach biotinylated aptamers to immobilized avidin. Several biosensors have been developed using aptamers [201] to detect cancer cells, [202] proteins, [203,204] and drugs. [205]

A common and easy functionalization procedure attaches a receptor with a directed orientation to the functional group of a SAM, Fig. 1.8c. A number of biosensors

using receptors attached to SAMs have been developed. [206] Functionalized SAMs can be used to attach a variety of receptors such as antibodies, [207] proteins [208] and bacteriophages. [209] In particular, bacteriophages are well understood viruses that are genetically engineered to express proteins at predetermined areas on their body. Bacteriophages are used to detect a wide variety of analytes, Fig. 1.8d. [210] SAMs with alkyl chains of different lengths can be mixed and assemble regularly on surfaces. Longer SAMs with functional groups attach the receptor. Shorter SAMs are spacers that increase the distance between receptors and reduce steric hindrance. Many sensors based on functionalized mixed SAMs have been developed such as sensors for *E. coli* bacteria [211] and PSA. [212] Polymer brushes are polymer chains grafted on one end to a surface. Simple or mixed polymer brushes can be polymerized on surfaces and used to attach biochemical receptors. [213]

Ion channels in cell membranes can be mimicked for specific antigen detection, Fig. 1.8e. [214] A lipid bilayer is formed on the surface of an impedance element. Membrane spanning lipids are functionalized with biotin, streptavidin and biotinylated antibody fragments. Mobile ion channels are functionalized with specific antigens to the antibody fragment, keeping the ion channel tethered far from their distant inner-layer partner. An antigen is introduced binding competitively to the antibody fragment, releasing the other antigen and opening the ion channel. Ions flow through the channel increasing the membrane conductance. This approach can be used with most types of receptors such as nucleic acids and antibodies. Dendrimers are spherical tree-like hierarchical molecules that can be used for multiple biological applications. [215] Dendrimers can be used to encapsulate receptors and increase signal and are incorporated into several sensors. [216] Dendrimers are mostly used to detect ions and to increase signal in biosensors. [217]

1.10.2 Receptor patterning and integration

There are many ways to pattern receptors and integrate them into microfluidic devices, Fig. 1.8f-k. Microcontact printing with inked PDMS stamps are used to pattern SAMs and proteins onto surfaces with sub-100-nm resolution. [225] By applying a controlled pressure onto a PDMS stamp, antibodies are accurately microcontact-printed into the bottom of

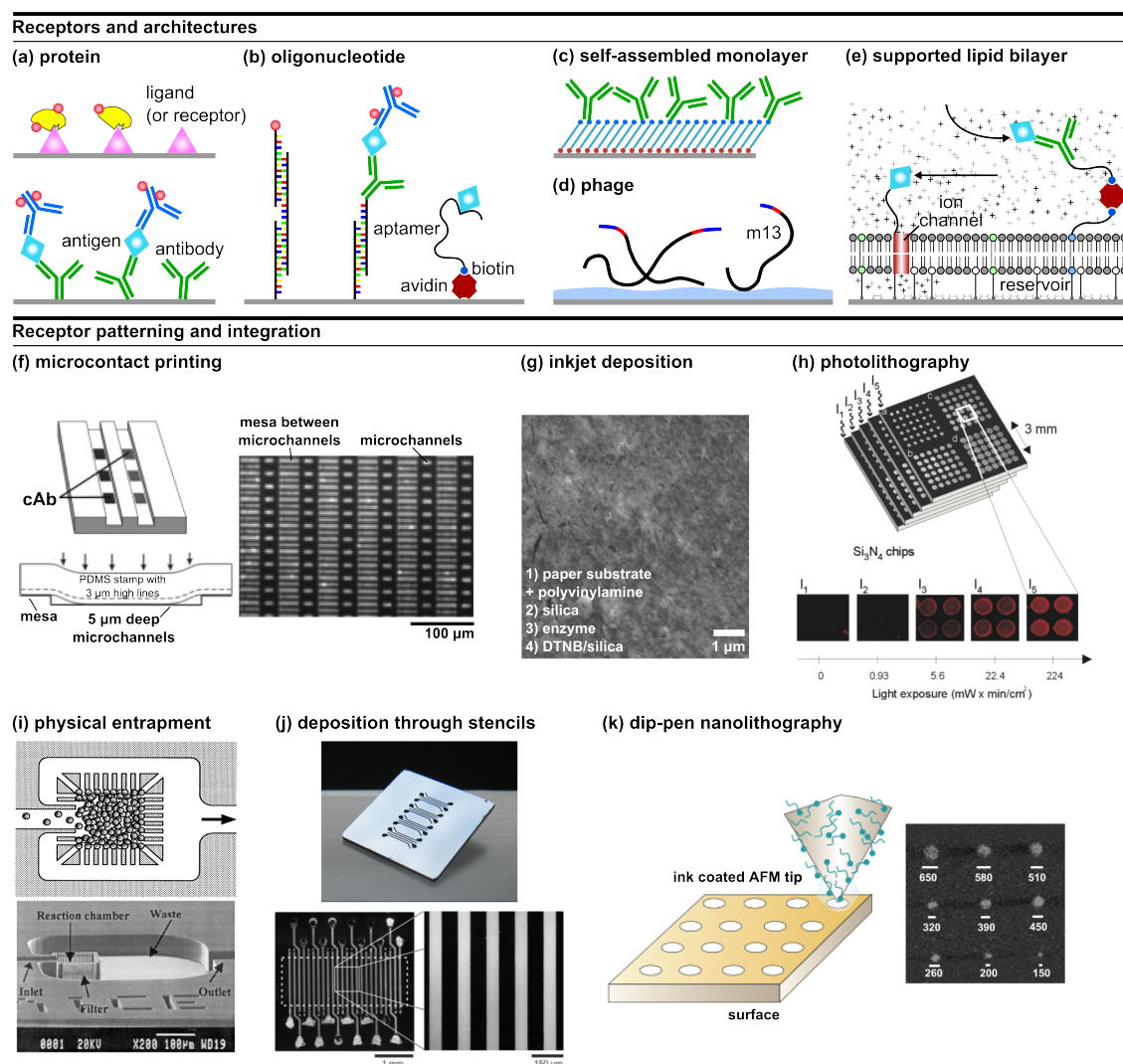


Figure 1.8: Receptors. Biological assays use receptors such as (a) proteins, (b) oligonucleotides, antibodies linked to oligonucleotide barcodes and aptamers, [198] (c) functionalized self-assembled monolayers, [208] (d) bacteriophages, and (e) supported lipid bilayers. [214] Receptors can be patterned and integrated into microfluidic devices using (f) microcontact printing of proteins inside microfluidic channels, (g) multilayer inkjet printing of enzymes between layers of silica and Dithiobisnitrobenzoate(DTNB)-doped silica, (h) antibody gradients of different densities depending on the intensity of light exposure to a photosensitive polymer, (i) micromachined filter pillars for bead capture, (j) stencil patterning of antibodies on PDMS, (k) dip-pen nanolithography to pattern protein dots with decreasing diameters depending on contact time. Reproduced with permission: (f) from [218], copyright 2005 Royal Society of Chemistry; (g) from [219], copyright 2009 American Chemical Society; (h) from [220], copyright 2002 American Chemical Society; (i) from [221], copyright 2001 Wiley-VCH; (j) from [222], copyright 2008 American Chemical Society; (k) from [223], copyright 2007 Macmillan Publishers, and from [224], copyright 2006 Wiley-VCH.

microchannels with aspect ratios lower than 1:6, Fig. 1.8f. [218] Immunoassays can then be realized on the microfluidics chips with integrated receptors.

Inkjet printing, contact and non-contact pin spotting are commonly used to deposit receptors on microarray slides. [226] Additives in spotting solution can increase signal and improve storage. [227] These techniques are routinely used for low-cost mass-fabrication of immunoassay strip tests. A variety of chemicals can be patterned in multiple steps to produce complex architectures. For example, an inkjet printer can be used to deposit enzymes that are covalently immobilized on paper within sol-gel-derived silica entrapments, Fig. 1.8g. [219] The density of receptors can be tailored based on the application. Proteins can be patterned using photolithography. Photosensitive polysaccharide-based polymer is photopatterned and antibodies of different densities are immobilized depending on the intensity of light exposure, Fig. 1.8h. [220]

Bead-based immunoassays are common in biochemistry laboratories. The surfaces of beads are functionalized with receptors to capture analytes and magnetic beads can be used for separation and concentration, or colorimetric gold nanoparticles or latex beads for colorimetric signal generation. Devices have been proposed incorporating beads into microfluidic devices to simplify manipulations. For example, micromachined filter pillars in a microchannel entrap functionalized beads, Fig. 1.8i. [221] Magnetic beads are retained in self-assembled chains in microchannels with varying cross-sections in the presence of a homogenous perpendicular magnetic field facilitating more efficient mixing and enhanced antibody-antigen interaction. [228] Assays and sequencing experiments can be performed with sequential liquid flows. Beads are assemblies of receptors that can increase the number of binding site in a given volume compared to detection regions on surfaces. For example, micropillars of hydrogel incorporating 1 μm antibody coated particles fabricated inside a microchannel can be used for multiplexed immunoassays for the detection of C-reactive protein, α -fetoprotein and PSA. [229]

The adsorption of proteins from solution onto hydrophobic surfaces can be exploited as a simple protein deposition method. [230] Antibodies can be patterned on PDMS using microfabricated capillary stencils, Fig. 1.8j. [222] Different antibody solutions are pipetted into independent loading pads and capillary channels to pattern dense lines of homogenous

monolayers of protein with widths of tens of micrometers. Alternatively, a microfluidic probe can pattern spots of proteins with dimensions of $50\ \mu\text{m}^2$. [231] The microfluidic probe scans a surface and has aspiration and injection apertures that hydrodynamically confine a flow of liquid in between the probe and the surface. The techniques described here pattern proteins under wet conditions thereby preventing drying artifacts happening when using inkjet technologies. PDMS was demonstrated as a viable biological substrate alternative to polystyrene (used for example in Petri dishes and microtiter plates for ELISA), with protein patterns maintaining biological activity for more than six months when stored in a dry state. [31] Other techniques can pattern receptors in extremely small areas. Dip-pen nanolithography scans an atomic force microscope tip imbibed in a protein ink [223] to pattern spots with diameters from 150 nm to 650 nm depending on the time of contact with the surface, Fig. 1.8k. [224]

1.10.3 Signal transduction

Biochemical recognition of analytes in a microfluidic chip must be transduced into a useable signal such as an optical or electrical signal. There are two main classes of signal transduction: labeled signal transduction techniques measuring a particle or molecule attached to a receptor, some of which are illustrated in Figure 1.9, or label-free techniques directly measuring physical effects caused by biochemical binding events, as shown in Figure 1.10.

1.10.4 Labeled detection of analytes

Optical detection is the simplest and most pervasive labeled detection method used to monitor biological tests and immunodiagnosics. Conventional lateral flow immunoassays for POC diagnostics often use colorimetric detection, Fig. 1.9a. There, the sample flows through membranes reaching deposited detection receptors labeled with particles (e.g. made of latex or gold). A complex between the analytes and detection receptors forms and continues through the membrane binding to a line of patterned capture receptors. As an example, a line of accumulated gold particles (appearing red when below 100 nm in diameter) functionalized with aptamers is visible when thrombin is captured in a lateral

flow immunoassay. [203] These tests are often qualitative and a line is visible only in the presence of analyte above a threshold concentration. A more advanced version of colorimetric detection is commonly used with ELISA in biochemistry laboratories as well as in clinical analyzers. In ELISA, a sample is pipetted into a plastic well coated with a receptor, after capture of the analyte by the receptor, the analyte is complexed with a detection antibody carrying enzymes. The analyte is detected by monitoring the generation of colored enzymatic products in the well. ELISA systems include many steps but may be simplified in miniaturized and automated devices. [232]

Direct detection of an analyte using fluorescence rather than monitoring an enzymatic activity is simpler. Here, the analyte is bound with a detection species carrying one or several fluorescent centers (e.g. fluorophores, naturally fluorescent protein, or quantum dots). Fluorescence detection is often used in microfluidic devices for POC diagnostics. [31] Areas as small as 30 by 100 μm^2 can easily be read with magnifying optics, filters and a charge-coupled device (CCD) camera, Fig. 1.9b. Detection areas can be densely packed in small areas and multiplexed to detect various analytes using fluorophores of different colors. [233] Fluorescence microscopy can detect fluorophores at a surface density well below 1 fluorophore/ μm^2 with a corresponding concentration of analyte in solution in the picomolar range. [32] A variety of technologies, which are constantly improving, have contributed to the high sensitivity of fluorescence spectroscopy such as light sources (mercury arc, xenon flash, gas and solid state lasers, etc.), labels (lanthanide chelate and cryptate, europium, etc.), and cooled CCDs. [234] A way to increase the signal of fluorescence assays is to increase the number of fluorophores attached to a receptor probe. Engineered optical paths can increase the amount of excitation or emission light. A device with a set of mirrors for transmission measurements and in coupling of light for fluorescent excitation was fabricated, Fig. 1.9c. [235] A second set of mirrors concentrates emitted fluorescence photons from a microchannel towards a detector on a surface. Waveguides can be integrated into PDMS devices for locally exciting fluorophores and collecting emitted photons to increase signal. [236] As an alternative to fluorophores, single walled carbon nanotubes can be used as multicolor Raman scattering labels for multiplexed detection. [237] Optical and fluorescent readers have been developed using a smartphone's integrated

camera. [238, 239]

Optical compact disk readers measure the intensity of reflected light from a laser diode on pits on the surface of a disk. Similarly, surface immunoassays using reflective labels of silver precipitation catalyzed by gold-labeled detection antibodies can be read with a standard optical disk reader, Fig. 1.9d. [240] In another approach, detection antibodies functionalized with magnetic nanoparticles are manipulated with a magnetic field on a surface onto which light is reflected to a camera, Fig. 1.9e. [241] The intensity of the light varies with the concentration of nanoparticles that frustrate total internal reflection on the surface. These methods can detect pM concentrations of protein in human serum.

There is no single best optical method due to different requirements in the optical paths needed to couple photons into the system and/or read them out, in addition to components of the chemical system that absorb or generate photons. In fluorescence detection, light intensity increases according to the inverse square of the distance ($1/d^2$). Excitation and collection of emitted photons should be done locally for increased signal. The optical path can be complex because it requires bright light of a given wavelength at the absorption peak of a fluorophore, magnifying optics and filters that might be difficult to miniaturize. However the complexity is in the reader and not in the number of fluid handling steps in the chip. In optical absorption and colorimetric detection, the signal increases with distance (d) according to the Beer-Lambert law. A long optical path is required to accumulate signal, making miniaturization difficult. Colorimetric detection requires a few micrometers depth on membranes or millimeters in liquid wells. Chemiluminescence is an alternative optical technique that depends on a chemical reaction between the sample, luminescent compounds (e.g. luminol, acridium esters, acridium sulphonamides) and enzyme reagents (e.g. peroxide and horseradish peroxidase). Unlike fluorescence, there is no need for light excitation, since light emanates from the luminescent molecules. This makes the optical path simple and chemiluminescence assays easy to miniaturize and integrate into microfluidic devices. [242] Chemiluminescence assays require several liquid handling steps for the introduction of multiple chemical reagents. Several sensitive laboratory analysis machines use pipetting robots to perform chemiluminescence assays. [243]

Electrochemical detection measures an oxidizable or reducible substance resulting in a

chemical reaction causing an electron flow on the surface of an electrode. Typically, three electrodes are involved: a working electrode where the reaction takes place, a counter electrode, and a reference electrode compensating for shifting electrical conductivity in the sample. The total charge is proportional to the local concentration of the analyte on the surface of the working electrode. For example, an immunoassay on an electrochemical sensor with adsorbed capture antibodies detects staphylococcal enterotoxin B using biotinylated antibody and horseradish peroxidase labeled streptavidin, Fig. 1.9f. [244] An array of working electrodes can be individually functionalized with capture antibodies for multiplexed detection. Proteins can be detected in picomolar concentrations but the process requires incubation and many fluid manipulation steps. Interestingly, by patterning enzyme-labeled receptors directly on working electrodes analytes such as glucose, lactate and oxygen, [245, 246] nucleic acid [247] and protein [248, 249] can be detected electrochemically in one-step.

Magnetic particles can be detected close to a surface. The dipole field from a magnetic particle decreases with the inverse cube of the distance ($1/d^3$) with the magnetic sensor. Therefore, in order to measure a strong signal, magnetic particles should be in close proximity to the sensor. There are several examples of using a magnetic signal to transduce biochemical recognition events. Among those, a recent work exploits the GMR effect to detect biotin labeled antibodies using streptavidin labeled magnetic particles, Fig. 1.9g. [250] The GMR sensor is an extremely sensitive transduction technique and it can detect multiplexed tumor markers at attomolar concentrations. However, using the GMR sensor requires several manipulation steps and it has not yet been implemented into a POC device.

1.10.5 Label-free detection of analytes

Label-free detection techniques measure an analyte binding directly on a sensor surface. These techniques require only a single capture receptor and can be very sensitive. Label-free detection can be prone to non-specific binding on the sensor surface. Measuring optical changes due to biochemical binding is perhaps the simplest label-free techniques. One example is a simple and sensitive method to quantify proteins or peptides captured on surfaces by measuring changes in the energy of interaction of liquid crystals with

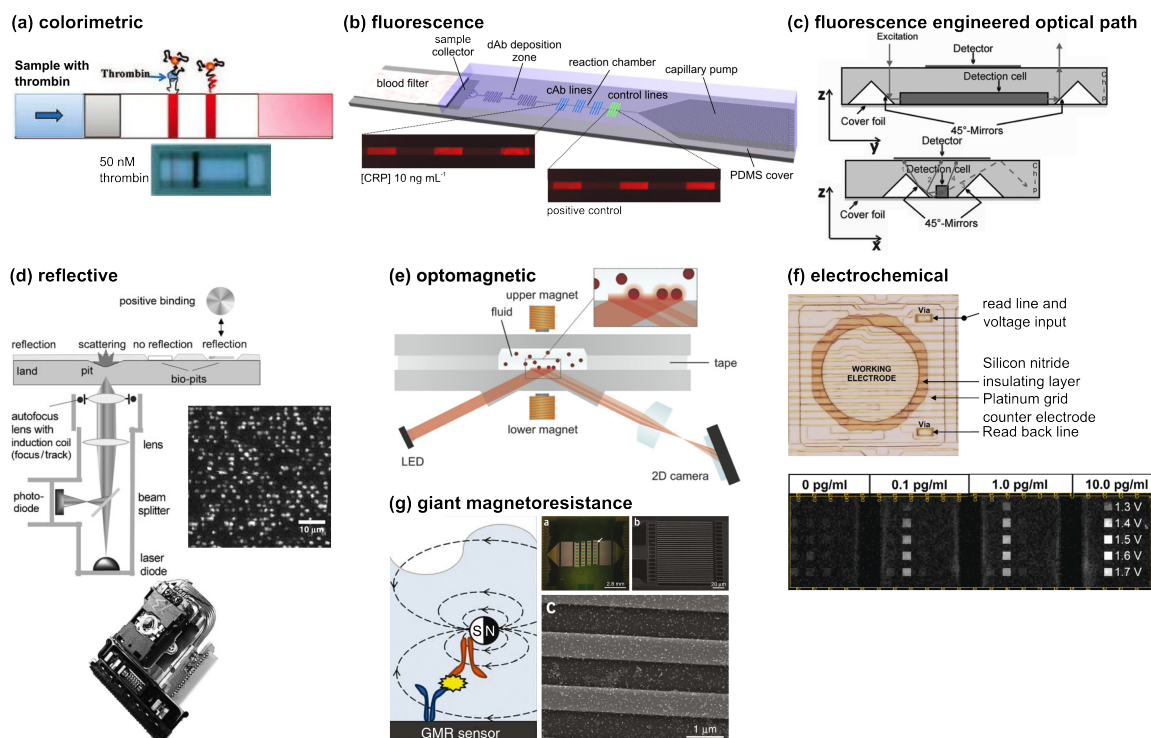


Figure 1.9: Labeled signal transduction. Biochemical binding can be transduced using labeled techniques such as (a) colorimetric particles, (b) fluorescence, (c) fluorescence in an engineered optical path, (d) particle reflection using a compact disk reader, (e) optomagnetic capture and reading, (f) amperometric reading, and (g) giant magnetoresistance. Reproduced with permission: (a) from [203], copyright 2009 American Chemical Society; (b) from [31] and (c) from [235], copyright 2009 Royal Society of Chemistry; (d) from [240], copyright 2006 Wiley-VCH; (e) from [241], copyright 2009 Royal Society of Chemistry; (f) from [244]; (g) from [250], copyright 2009 Macmillan Publishers.

biomolecule bound surfaces, Fig. 1.10a. [251] Polarized light images are captured and changes in polarization angles are used to calculate the anchoring energy of liquid crystals and the concentration of analytes on the surface. Concentrations of antibodies from 10 pM to 100 nM can be measured and multiplexed on a single surface.

Surface plasmon resonance (SPR) is commonly used in laboratories to observe biochemical binding in real time and to determine binding constants. In SPR, a light beam is reflected on the bottom of a thin metal film and reflected light is detected. Molecules binding onto the metal film change the local index of refraction and the resonance of surface plasmons in the metal, leading to a change in the angle and wavelength of reflected

light. One such SPR sensor consists of 264 individually addressable chambers isolated by microvalves for high throughput quantification of binding affinities, Fig. 1.10b. [252] SPR sensors are increasingly being miniaturized into POC devices. [253] SPR sensors can be expensive to fabricate since they require metal deposition and microfabrication. High quality optics are required and temperature control since the signal varies with temperature, but these can be integrated into a separate reader.

Optical grating patterns of periodic lines of receptors with interspersed passivated lines can be used to quantify biochemical binding. To be sensitive, optical gratings are fabricated with a typical depth modulation and a period below about 20 nm and 500 nm respectively. A grating coupler sensor measures changes in light modes and angle from light passing through or reflected on a grating on a planar waveguide. [254] A diffraction based sensor measures the attenuation of light by a surface grating on a planar waveguide. [255] Guided light intensity, mode and angle vary depending on the step depth that increases with the binding of analytes to receptor lines. The complexity of fabricating and functionalizing precise nanometer patterns is a limit of optical grating biosensors. Alternatively, a microtoroid resonator biological sensor can detect an optical resonance shift from a single molecule binding event. [256] Similarly, waveguides with functionalized optical resonant cavities can detect the specific binding of analytes, Fig. 1.10c. The binding of analytes increases the refractive index and the wavelength of resonating light of the optical resonant cavities causing a red-shift of the output spectrum. [257]

Simple electrical transduction devices are common and sold on the market. For example, glucose is detected amperometrically in blood from a finger prick for the management of diabetes. [258] An alternative is electrochemical impedance spectroscopy. [259] More integrated electrical transduction devices such as ion sensitive FETs, [260,261] nanowires, [262–267] and carbon nanotubes [204,268–270] are known but it is hard to imagine their evolution from research to low-cost disposable devices. These devices are expensive to fabricate, complex and the integration of specific receptors for analytes onto sensing areas is challenging. One example of electrical transduction is the detection of analytes using nanowire FETs, Fig. 1.10d. [271] Charged analytes binding to receptors immobilized on the surface of the channel change the electrical conductance between the source and the

drain. These sensors are sensitive to non-specific binding and changes in the local ionic charge of the sample. Reliability and stability are problems that can be partially mediated with reference electrodes.

QCM is a common mechanical transduction technique used in laboratories. [272, 273] Analytes binding to a functionalized quartz crystal resonator surface are measured as a resonant frequency shift. A miniaturized alternative is the microcantilever, Fig. 1.10e. [274] Microcantilevers can operate in two modes. In resonant detection, a change in mass detects analytes that are typically particles such as bacteria and viruses. In deflection mode, the cantilever bends due to a surface stress caused by the uniform binding of analytes that are typically smaller molecules such as proteins, nucleic acids or chemicals. Microcantilevers are prone to similar problems as electrical transducers, namely they are sensitive to non-specific binding and exhibit reliability and stability issues. Another alternative are surface acoustic wave sensors (SAW) that measure a change in the velocity of an acoustic wave along a surface with bound analytes. [275]

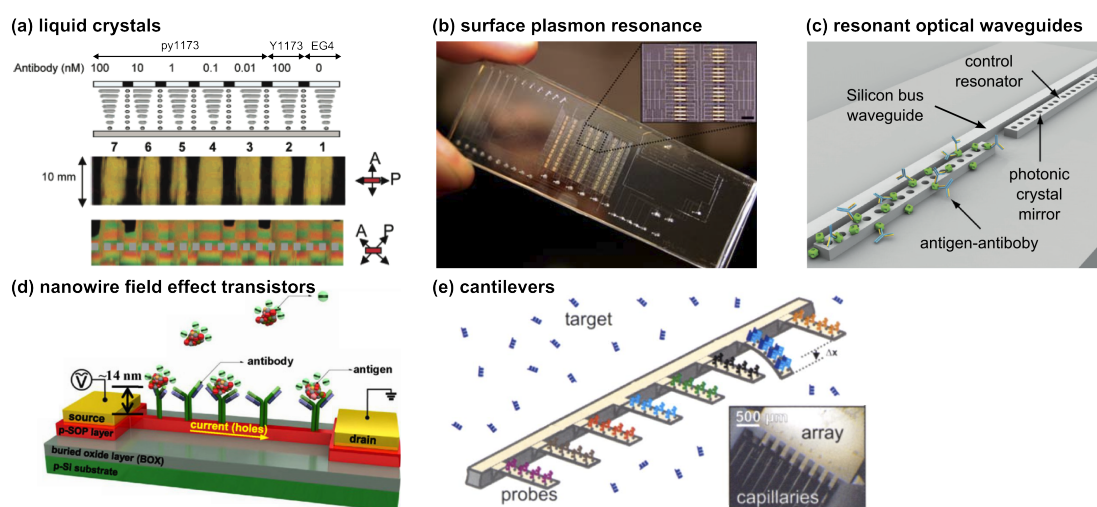


Figure 1.10: Label-free signal transduction. Biochemical binding can be transduced using label-free techniques such as (a) liquid crystals, (b) surface plasmon resonance, (c) resonant optical waveguides, (d) nanowire field effect transistors, and (e) cantilevers. Reproduced with permission: (a) from [251], copyright 2007 American Chemical Society; (b) from [252], copyright 2010 Royal Society of Chemistry; (c) from [257], copyright 2009 Royal Society of Chemistry; (d) from [271], copyright 2010 Elsevier; (e) from [274], copyright 2002 National Academy of Sciences, USA.

Several of these label-free detection methods are very sensitive. However, proven reliability is lacking because events such as a change in the ionic strength of the surrounding medium, non-specific deposition on the sensing surface and change in temperature, compromise the long term stability of the sensors. Sometimes the original publication documents these sensitive sensors as not reliable enough for POC diagnostics. For example, a complementary metal-oxide-semiconductor (CMOS) nanowire immunoassay sensor can detect proteins at 100 fM concentrations but the signal is only stable for 30 seconds. [262] Most POC devices for immunoassays use labeled detection of analytes where current or light/fluorescence is measured. This of course requires the integration of reagents to the device as well as integrating a cascade of chemical events (e.g. dissolution, binding, rinsing) that also must be timed properly.

1.11 From components to working devices

Microfluidic components need to be integrated into complete functioning devices. This is where good ideas and deep understanding of the underlying physics of microfluidic systems need to be compatible with packaging techniques, practical form factors and overall manufacturability to realize successful end products. The attributes and performances of microfluidic devices in research laboratories are rarely those of final mass-fabricated products.

1.11.1 Sample to chip interface

It was early recognized that while microfluidic devices are excellent at manipulating microliter and nanoliter volumes of liquid by the means of microfabricated structures, a great challenge is to load such a small sample onto microfluidic devices. This is often referred to as the world-to-chip interface challenge. [276, 277] A sample from a patient can easily be hundreds of microliters and it must be accurately positioned on the sample receiving area of a microfluidic chip. We think that pipetting about 10 microliters of sample onto a chip is the “sweet spot”. This volume can be accurately measured and delivered using a conventional, disposable pipette. This volume is not large and keeps some advantages of microfluidic technologies in terms of sample volume reduction

without imposing specific skills/training from the operator or requiring dosing peripheral instrument. Once on the chip, the sample can be filtered, metered and drawn for analysis. If particular fittings, connections or interfaces are needed to accommodate the sample, these elements need to be packaged onto the chip and this may represent a significant cost of fabrication in the case of disposable diagnostic devices. Some examples of reversible electrical and fluidic connections with microfluidics chips are based on springs [278] and magnets. [279] Alternatively, a microfluidic chip can be used to dispense nanoliter volumes of sample onto another microfluidic chip. [280] As a rule of thumb, to ease chip handling a diagnostic device should not be smaller than half the size of a credit card. The region where the sample is loaded should not come into contact with surfaces from the reusable peripheral reader in order to avoid contamination of subsequently used chips.

1.11.2 Device assembly and fabrication

The review by Becker *et al.* [40] provides an in depth assessment of the techniques for fabricating microfluidic devices in plastic. Mold injection is the method of choice for the fabrication of disposable microfluidic devices owing to its throughput, potential in resolution and ability to form 3D structures. However, what can be achieved on a mold in terms of resolution, aspect ratio and fill factor are not necessarily identical in the mirror image fabricated chip. Chips in the “lab-on-a-CD” format can use mass fabrication techniques for CDs and benefit from economies of scale. Sealing microfluidic chips using adhesives sensitive to pressure, heat, or light is frequent [281] but can be a problem if biological species are integrated into a chip. Ultrasonic welding and adhesion following surface activation using plasma treatment are also available. [282, 283] Alternatively, a microfluidic chip can be inserted into a housing cover without complete sealing. [98] In this case, a lower microfluidic component directs the flow of liquid using three wetting walls and the cover provides mechanical protection and minimizes evaporation of sample from the chip.

1.11.3 Temperature control

It is crucial to control temperature affecting reaction conditions and the stability of the sensor. This is especially important for nucleic acid tests with the need for controlled dehybridization and hybridization. Approaches to heating in PCR microfluidic chips were reviewed by Zhang *et al.* [80] Heaters can be integrated on the chip or on the peripheral reader, although the costs of disposable devices can be minimized by keeping the complexity in the reader. A common approach is resistive heating. For example, a resistive circuit can be made of a microchannel injected with a silver-filled epoxy. [284] On-chip resistive heating and off-chip thermoelectric cooling can be used for thermal cycling. [285] Contact-less approaches simplify the interface between the chip and the reader. For example, inductive heating can be integrated on a chip with microfabricated coils, [153] infrared heating with integrated thermocouples for temperature regulation, [286] and radio frequency microwave heating with temperature dependent fluorescent intensity measurements. [287] Heating and cooling can be done with Peltier elements on or off-chip [288–290]. Lasers can be used to heat precise areas on microfluidic chips. [291–293] An approach used for cooling electronic chips consisting of circulating liquid in microchannels can be used to heat and cool chips using convective heat transfer. [294–296] Multiple temperature zones on one chip are easier to implement in flow path materials with low thermal conductivity such as polymers instead of materials with high thermal conductivity such as glass or silicon.

1.11.4 Hardware and Software

Until recently, any electrical component included in a diagnostic device would have been considered non-disposable. Now, many components that can be key for POC devices have had their cost divided by more than 100 fold in the last 10 years. This is the case of memory modules, processors, image sensors (CCD, CMOS), screens (based on liquid crystal displays or even organic light emitting diodes). Considering peripheral equipment, a number of portable readers to measure signal from microfluidic devices have been developed. [297–299] These readers include electronics for detection, signal processing, and signal transmission that operate on battery power. Important metrics for these

electronics components are high performance with low power consumption. Sarpeshkar wrote an informative book on low power electronics for biomedical applications. [300] Readers can operate as standalone units or connected to a computer. Barcode or readers for radio frequency identification (RFID) can identify information from the diagnostic chip inserted in the device such as the chip number, name of the analyte, lot number and the date of purchase. The reader can transmit data to an electronic health record (EHR) database using a wired connection (such as a universal serial bus or a proprietary connector) or wireless connection (such as Bluetooth[®], Wi-Fi[™] (IEEE 802.11) or GSM). Diagnostic information should be kept private using encryption with at least a 128 bit cipher algorithm encryption key (e.g. AES) and especially when transferring data wirelessly.

Diagnostic and health monitoring devices can be connected with a smartphone providing the power, processing, display and data transfer functions. The use of chips for immunodiagnosics is not as ubiquitous as glucose monitoring devices are. However, glucose monitoring devices give a good perspective of what immunodiagnostic chips could become. The Jazz[™] system from AgaMatrix is an example of a blood glucose test strip and reader transmitting test results via Bluetooth[®] or a wired connection to a smartphone. Similar devices for glucose monitoring are available such as the Sanofi Aventis iBGStar[™] and the Johnson & Johnson Lifescan OneTouch[®]. Some examples from academic developments are a POC device and reader connecting to a smartphone [301] and microscopy using a smartphone camera. [239] Mobile health devices are emerging as personal health data interfaces to the internet and offering opportunities for disease management and prevention beyond centralized healthcare. Personally controlled online health data services such as Google Health and Microsoft HealthVault[™] give consumers more control on their healthcare. [302, 303] Historically, electronic health systems have been regionally and independently developed (e.g. in each state or province). The isolated and closed development of specific and incompatible health applications has limited their development and widespread use. There is rising interest in developing health information systems using open architectures, common protocols and file formats (such as those that enabled the internet) in order to prevent fragmented and duplicated work, or monopoly situations (such as with operating systems and office productivity software). [304, 305]

1.12 Outlook

Microfluidic devices are often first developed in research laboratories by integrating complex functions, materials and concepts into a single device. Following the original prototype, efforts are invested to simplify the technology while preserving its performance. We reviewed several interesting examples in which well-developed technologies are revisited in ingenious ways using inexpensive materials (e.g. paper), [36] low cost manufacturing and packaging methods, and pervasive imaging/detection devices (e.g. cameras from consumer electronics) [238] to get diagnostic devices out of laboratories.

One question that arises is if we already have most of the technology and know-how to make these devices, why isn't POC diagnostics a killer application for microfluidics? The development and adoption of microfluidic based POC diagnostics is impacted by the segmented nature of the diagnostics market, major existing clinical technologies, fierce competition in the market, strong regulation from public health agencies, patents on reagents (such as antibodies and biomarkers) and on technology components. The reimbursement of diagnostic devices, their acceptance by medical personnel (users), business models that are rarely intuitive, large geographical disparities and medical practices add to the complexity of deploying new technologies. A solution to all these challenges is probably to identify key analytical needs in the life sciences and to create reference applications involving microfluidic technologies. Changes are accelerating thanks to the steadfast pace of development of technology. Decades of basic research in biology, chemistry, materials engineering and information technology can now be harnessed in microfluidic devices to improve diagnostics, prevention and therapeutic monitoring.

In our opinion, microfluidic diagnostics devices are at the brink of supporting decentralized testing. These devices will enable patients to monitor themselves outside of the hospital and communicate results with clinicians, ultimately giving patients a greater control of their own healthcare and access to their personal health data. In addition, these devices may also support the emergence of personalized medicine in which treatments are tailored to the genetic profile of patients and/or to their specific metabolism of drugs. [306] For example, these devices might offer an accurate and convenient method for monitoring

drug efficacy, drug metabolism and drug interactions in the plasma of patients. [307] We believe that a wide library of microfluidic components have been developed, which can be combined into successful diagnostics devices. Now, more than ever, microfluidic devices will be evaluated based on their direct impact and usefulness to improve the healthcare of patients.

2

Toward one-step point-of-care immunodiagnostics

2.1 Preface

Point-of-care diagnostics will strongly benefit from miniaturization based on microfluidics because micro-fluidics integrate functions that can together preserve valuable samples and reagents, increase sensitivity of a test, and accelerate mass transport limited reactions. But a main challenge is to incorporate reagents into microfluidics and to make microfluidics simple to use. Here, we integrate microfluidic functional elements, some of which were developed earlier, and reagents such as detection antibodies (dAbs), capture antibodies (cAbs) and analyte molecules for making one-step immunoassays: the integrated device only requires the addition of sample to trigger a cascade of events powered by capillary forces for effecting a sandwich immunoassay that is read using a fluorescence microscope. The microfluidic elements comprise a sample collector, delay valves, flow resistors, a deposition zone for dAbs, a reaction chamber sealed with a polydimethylsiloxane (PDMS) substrate, and a capillary pump and vents. Parameters for depositing 3.6 nL of a solution of dAb on the chip using an inkjet are optimized and the PDMS substrate is patterned with analytes, which provide a positive control, and cAbs. Various storage conditions of the

patterned PDMS are investigated for up to 6 months revealing that storage with a desiccant preserved at least 51% of the activity of the cAbs. C-reactive protein (CRP), a general inflammation and cardiac marker, is detected using this one-step chip using only 5 μL of human serum by measuring fluorescent signals from $30 \times 100 \mu\text{m}^2$ areas of the PDMS substrate in the wet reaction chamber. The one-step chip can detect CRP at a concentration of 10 ng mL^{-1} in less than 3 min and below 1 ng mL^{-1} within 14 min. The work presented here may spur the adoption of fluorescence immunoassays using capillary driven microfluidics and PDMS substrates for point-of-care diagnostics. This chapter is based on Toward one-step point-of-care immunodiagnostics using capillary-driven microfluidics and PDMS substrates, L. Gervais and E. Delamarche, *Lab on a Chip*, 9, 3313-3452, 2009.

2.2 Introduction

Point-of-care diagnostics are tests administered directly at the side of the patient. Small sample volumes can be analyzed and results are obtained rapidly. Devices used for point-of-care diagnostics are portable, low cost and easy to use. They can be used by health care professionals in hospitals as an alternative or complement to clinical analysers. Medical staff and patients can also use point-of-care diagnostics outside of the hospital, in the field, and in the developing world. [308, 309] They are often called one-step assays, or one-handling step assays, a name which refers to the fact that results can be obtained simply after the introduction of sample onto the device.

Point-of care diagnostics are versatile. They can be used in a variety of situations such as to measure disease markers, [15, 310–313] to monitor therapies, [314] and to detect chemical and biological hazards. [315] Point-of-care diagnostics are especially useful at assessing health conditions that can rapidly occur and worsen. For example, the rapid diagnosis of heart problems is crucial to administer the appropriate treatment and to increase the chances of survival of the patient. In this case, the measure of the concentration of cardiac markers in blood is used to assess the cardiac risk profile of patients, the presence of myocardial injury and to predict the survival rate after a myocardial infarction. [316,317] Monitoring the evolution of the disease requires the analysis of cardiac markers to be performed periodically. For these reasons, there is a need for fast and accurate point-of-care

devices for detecting cardiac markers and more generally for detecting markers relevant to diseases that can evolve rapidly and in varying circumstances.

Many of the available point-of-care devices on the market are based on immunoassays. Immunoassays are a common technique used to detect antigens. Immunoassays for diagnostics are available in a variety of different formats ranging from the large high throughput clinical analysers confined to central laboratories to portable single use lateral flow tests. Clinical analysers measure the concentration of antigens and can measure several antigens sequentially. They require large sample volumes of a few hundred microlitres and provide a time to result of up to an hour. The simplest and most commercialized point-of-care tests on the market follow the principles of lateral flow assays in which an antigen is detected to be above a certain threshold. Lateral flow tests are low cost, portable and require a few tens of microlitres of sample. One such lateral flow test that has become ubiquitous is the pregnancy test. [182]

There are several examples of successful point-of-care diagnostics devices found on the market. It is common to employ an external reader for measuring and recording the signal when accurate tests are sought. The i-STAT[®] [318] is a portable analyser that uses disposable cartridges. Each cartridge performs an immunoassay that quantitatively detects one cardiac marker. The Biosite[®] Triage[®] [319] is capable of multiplexed quantitative detection of 3 cardiac markers within 15 minutes using 150 μ L of sample. [320] Amic has developed a lateral flow immunoassay device that fills using capillary forces between micropillars. [98, 321] Multiplexed immunoassays have been developed on paper patterned into millimetre wide channels. [322] It would be beneficial to further expand these technologies using even less volume of sample, increasing the sensitivity and accuracy, and measuring more analytes in parallel.

We suggest that a tight control over the volumes and flow rates of sample moved through microfluidic de-vices gives the opportunity to realize these goals. Our previous work has put emphasis on the development of microfluidics for immunoassays in which capillary forces are used to move liquids in microstructures and anti-bodies patterned on PDMS provided the specific receptors for the analytes. [183, 233] In this chapter, we show the integration of concepts dealing with capillary valves, capillary pumps, the

accurate patterning of cAbs to form capillary driven microfluidics for effecting one-step immunoassays.

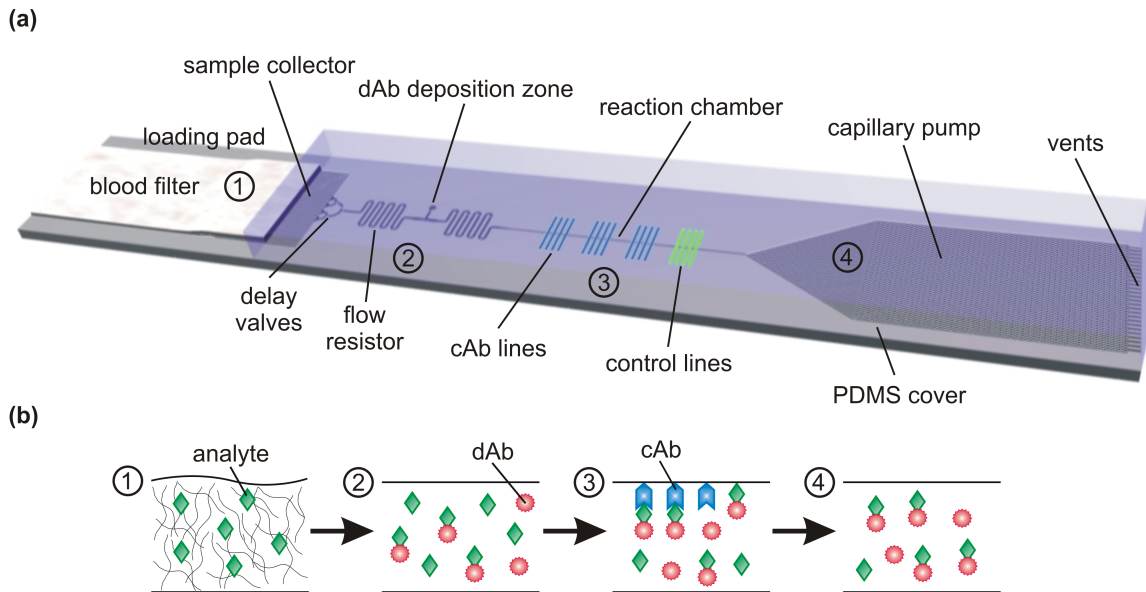


Figure 2.1: Concept of a capillary-driven microfluidic chip for effecting immunoassays with one step. (a) A series of functional microfluidic elements are implemented onto the chip for performing immunoassays. (b) The position of and interaction between the analyte, dAbs and cAbs are illustrated along different parts of the chip (see text for details). The PDMS is planar and patterned with lines of cAbs and antigens for the control lines. The Si chip has the loading pad, the sample collector, the delay valves, the dAb deposition zones with dAbs, the reaction chamber, the capillary pumps and the vents.

The strategy for implementing the concept of the one-step immunoassay chip is illustrated in Fig. 2.1a. The interaction of analyte, dAbs and cAbs throughout the microfluidic chip is shown in Fig. 2.1b. Blood or human se-rum sample is introduced on the blood filter. Cellular components of the sample, such as red blood cells, white blood cells and platelets, and debris are retained by the blood filter membrane and plasma is drawn in the sample collector and into a microfluidic channel. The sample reaches the dAb deposition zone where the dAbs reconstitutes and binds to the analyte. The dAb deposition zone can be a circular microwell or a recessed microchannel for accommodating dAbs spotted there using an inkjet. The analyte-dAb complex is transported by the moving liquid through the reaction chamber and binds to lines of cAbs on the PDMS cover. Excess dAbs binds to positive control lines of patterned analyte on the PDMS.

The sample containing excess analyte and dAbs is drawn by the capillary pump, which largely determines the flow rate and total sample volume used for the immunoassay. A few minutes after the introduction of the sample on the chip most of the dAbs has either bound to cAbs via the analyte, bound to the analyte forming the lines for positive control, or was transported to the capillary pumps. In principle, the background fluorescence from dAbs should be low enough to measure the specific detection of analyte on the capture areas, at the intersection of the lines of cAbs and reaction chamber. They can be measured through the PDMS using an external fluorescence reader.

2.3 Experimental

2.3.1 Chemicals and biochemicals

PBS tablets (Sigma-Aldrich, St. Louis, MO) were reconstituted in water that was purified using the Simplicity 185 system (Millipore, Billerica, MA) and filtered with a 0.20 μ m syringe filter (Sartorius, Epsom, U.K.). Bovine serum albumin (Sigma-Aldrich) was dissolved at a concentration of 1% w/v in PBS. Ethanol (puriss \geq 99.8%, Fluka, Sigma-Aldrich) was used for cleaning Si chips and PDMS. PDMS Sylgard 184 prepolymers (Dow Corning, Midland, MI) were mixed at a ratio of 1:10 curing agent to polymer using a DOPAG mixer (Cham, Switzerland), poured onto planar polystyrene Petri dishes (Greiner BioOne), and cured overnight in an oven at 60°C. Cured PDMS was 3 mm thick and cut to the desired size using a scalpel. cAbs were patterned on the surface of the PDMS that had been in contact with the Petri dish. [222] The blood filters were GF Vivid[®] plasma separation membranes (PALL, East Hills, NY). Human CRP (8C72), anti-CRP (4C28-C2, 4C28-C6), and human CRP-free serum (8CFS) were purchased from HyTest (Turku, Finland). Human CRP-free serum was spiked with human CRP to the desired CRP concentration. The anti-CRP-C6 Abs were labeled using an Alexa Fluor 647 labeling kit (Invitrogen, Carlsbad, CA). The fluorophore per protein ratio was measured to be from 3.6 to 3.9 using an Eppendorf BioPhotometer (Hamburg, Germany). Si chips were treated with 1% w/v Pluronic[®] F108 (BASF, Ludwigshafen, Germany) in purified water. PDMS substrates patterned with cAbs were stored with Sorb-IT silica gel desiccant packets (Sud

Chemie, Munich, Germany). Inkjet glass pipettes were cleaned with an alkaline solution of 2% v/v Hellmanex II (Hellma, Mullheim, Germany) in purified water. The solution of dAbs was composed of $250 \mu\text{g mL}^{-1}$ Alexa 647 labelled anti-CRP-C6, 68 mg mL^{-1} D-(+)-Trehalose (Fluka) and $700 \mu\text{g mL}^{-1}$ L-phenylalanine (Fluka) in PBS.

2.3.2 Chip fabrication and preparation

The microfluidic chips and stencil templates were microfabricated on 4-inch Si wafers (Siltronix, Geneva, Switzerland) using photolithography and photoplotting polymer masks (Selba, Versoix, Switzerland). The microfluidic chips were fabricated by first growing a $0.5 \mu\text{m}$ thick layer of SiO_2 onto a Si wafer using a wet oxidation process in a thermal furnace (TS-6304, Tempres, Vaassen, The Netherlands). Hexamethyldisilazane (HMDS) was spin coated onto the wafer. Photoresist (AZ 6612, MicroChemicals, Ulm, Germany) was spin coated at 4000 rpm to a thickness of approximately $1.6 \mu\text{m}$ and soft baked for 1 minute at 110°C . The first layer mask containing all the features of the microfluidic chip was brought into soft contact with the resist-coated wafer and exposed to a 6.5 mW/cm^2 ultraviolet light for 15 s using a mask aligner (MA/BA6, Süss Microtech, Garching, Germany) and developed. SiO_2 was etched from the features uncovered by the photoresist in buffered hydrofluoric acid (BHF, 7:1 volume ratio of 87.5% NH_4F in water to 12.5% HF in water; danger). The resist was stripped and HMDS was spin coated onto the wafer. A layer of AZ 4562 (MicroChemicals, Ulm, Germany) was spin coated at 4000 rpm to a thickness of approximately $7 \mu\text{m}$ and soft baked for 1 minute at 110°C . The second layer mask containing all the features of the microfluidic chip except the reaction chamber was photoexposed for approximately 45 s and developed. Deep reactive ion etching (AMS-200SE, Alcatel Micro Machining Systems, Annecy Cedex, France) was used to etch the second layer features in Si to a depth of $160 \mu\text{m}$. The resist was stripped and the first layer features, containing all the features of the microfluidic chips, were etched to a depth of $20 \mu\text{m}$ using reactive ion etching. The total depth of features was $20 \mu\text{m}$ in the reaction chamber and $180 \mu\text{m}$ elsewhere. The remaining SiO_2 was removed in buffered hydrofluoric acid in approximately 5 minutes.

Deep reactive ion etching was used to transfer features and etch through Si wafers

to make the stencils. Si wafers with stencils were diced into $12 \times 12 \text{ mm}^2$ individual templates having 4 groups of 4 stencils. Each stencil comprised of a loading pad connected to a 2-mm-long and $100\text{-}\mu\text{m}$ -wide line etched through the wafer. The microfluidic chips were cleaned using an O_2 -based plasma (Tepla microwave-plasma system 100, PVA Tepla, Asslar, Germany) for 2 min at 200 W and 0.7 Torr. The microfluidic chips were placed in a 1% solution of Pluronic[®] F108 for 30 min, rinsed with purified water and dried under an N_2 stream. The chips were stored in a dark and dry environment and used within 4 h.

2.3.3 Capture antibody patterning on PDMS

Stencil templates were cleaned using an O_2 -based plasma. Stencils were fabricated using single side polished Si wafers. The polished side of the stencils was placed onto PDMS substrates. Stencils were filled with a solution of $125 \mu\text{g mL}^{-1}$ anti-CRP-C2 in PBS for a 15 min long deposition of cAbs on PDMS at room temperature. To prevent evaporation of the cAbs in the stencils, the deposition was undertaken in a humidity chamber consisting of a small Petri dish with sheets of clean room paper soaked in water on the bottom. The stencils were rinsed under a stream of PBS and purified water. PDMS substrates were separated from the Si stencil template using tweezers in the presence of a blocking solution of bovine serum albumin where they were incubated for 15 min. The regions of the PDMS not patterned with cAb were passivated with bovine serum albumin. The PDMS substrates were then rinsed with a stream of PBS, purified water and dried under a stream of N_2 . Templates were rinsed in purified water, ethanol, and cleaned with O_2 plasma before reuse. [222] The PDMS substrates were placed onto microscope slides with the patterned side facing upwards. The substrates were stored in N_2 purged 50 mL Falcon conical tubes (BD Biosciences, San Jose, CA) in the dark. A Silica gel packet was inserted into some of the conical tubes. These substrates were used for the study of the lifetime of cAbs on PDMS or for exploring the performances of one-step immunoassays, in which case they were used within 3 days.

2.3.4 Study of the lifetime of cAbs on PDMS

A PDMS substrate having lines of cAbs was placed on a microfluidic chip with the lines of cAbs perpendicular to the reaction chambers. The chip covered with the PDMS substrate was then placed into a humidity chamber to prevent evaporation of the samples in the loading pads. These immunoassays were performed on multistep chips that had 6 independent flow paths. [222] One μL of human serum spiked with CRP was pipetted into the loading pads, followed by 0.5 μL of 250 $\mu\text{g mL}^{-1}$ dAbs and 1 μL of CRP-free human serum for rinsing. After 14 min, the PDMS was removed from the microfluidic chip, rinsed under a stream of PBS, rinsed with purified water, dried under a stream of N_2 , and the fluorescent signals on the PDMS were imaged in the fluorescence microscope.

2.3.5 One-step immunoassay

The solution of dAbs was deposited in the microfluidic chips using an Autodrop MD-P-705-L (Microdrop, Norderstedt, Germany) inkjet printer and a AD-K-501 piezo-driven pipette having a nozzle of 70- μm in diameter. The pipette was first cleaned with Hellmanex[®] II (Hellma, Mllheim, Germany) alkaline solution for two hours and rinsed with purified water in order to remove possible residues from previous experiments. The inkjet pipette nozzle was positioned over the detection antibody deposition zone where 20 drops of 180 pL each were deposited for a total of 3.6 nL of detection antibody solution. Solutions of dAbs can be inkjet deposited on the surface of a chip next to the circular well. In this way, there are dAbs in the channel and on the surface of the chip between the Si and PDMS substrate. This further slows down the redissolution of dAbs in the sample and increases the detection signal. This deposition method was used for immunoassays on the one-step chips. After deposition the microfluidic chip was stored in the dark and used within 4 hours. The conditions for jetting the drops and their geometry were optimized using a camera and stroboscopic viewing system embedded in the inkjet instrument. Ten μL of human serum was used to characterize the filling behaviour of the chip. A blood filter was placed in such a way that the interface with the chip covered about half of the serum collector. A paper clip was used to provide sealing pressure between the filter and the serum collector with the other half and the rest of the microfluidic chip covered with a piece

of bovine serum albumin passivated PDMS. For the one-step experiments one or several PDMS pieces, one of which having lines of cAbs, were placed on the microfluidic chips. The microfluidic chip covered with PDMS was then placed on the stage of a fluorescence microscope. The immunoassays started when 5 μ L of human serum were pipetted into the loading pad of a microfluidic chip. Fluorescence in the reaction chamber was monitored directly by viewing through the PDMS. After the experiments, the microfluidic chips were rinsed with purified water, ethanol, dried under a stream of N₂, and cleaned in the UV-ozone reactor and reused in further experiments.

2.3.6 Instrumental setup and data acquisition

High-resolution fluorescence micrographs (using 10 \times objectives) were obtained using a fluorescence microscope (Eclipse 90i, Nikon, Japan). Excitation of fluorophores was done using a 100 W halogen lamp and images were taken using a black and white CCD camera (DS-1QM, Nikon) cooled to -30°C. The software NIS-Elements (Nikon) was used for analysis of the fluorescence images. Videos were composed by joining a series of time-lapse images.

2.4 Results and Discussion

An example of a one-step microfluidic chip is shown in Fig. 2.2. This chip is in Si but other materials can be used. This layout can be replicated in materials such as cyclic olefin copolymer, poly(methylmethacrylate) and polycarbonate using mold injection or hot embossing with a low cost of fabrication and to make the chips disposable after each test. The microfluidic chip was treated with an aqueous solution of Pluronic® F108. Pluronic® F108 is a block copolymer of the formula HO-(CH₂CH₂O)₁₃₃-(CH(CH₃)CH₂O)₅₀-(CH₂CH₂O)₁₃₃-H which is used as a surfactant and a wetting agent. It is not the goal of this chapter to investigate in detail how Pluronic® F108 interacts with hydrophobic and hydrophilic surfaces [323] but we noticed that after microfluidic chips were cleaned with oxygen plasma and treated with aqueous Pluronic® F108, the contact angle of human serum with the chip increased from 0° to 20° (with a receding contact angle of 0°). This characteristic was highly reproducible and the surface was stable for at least 4

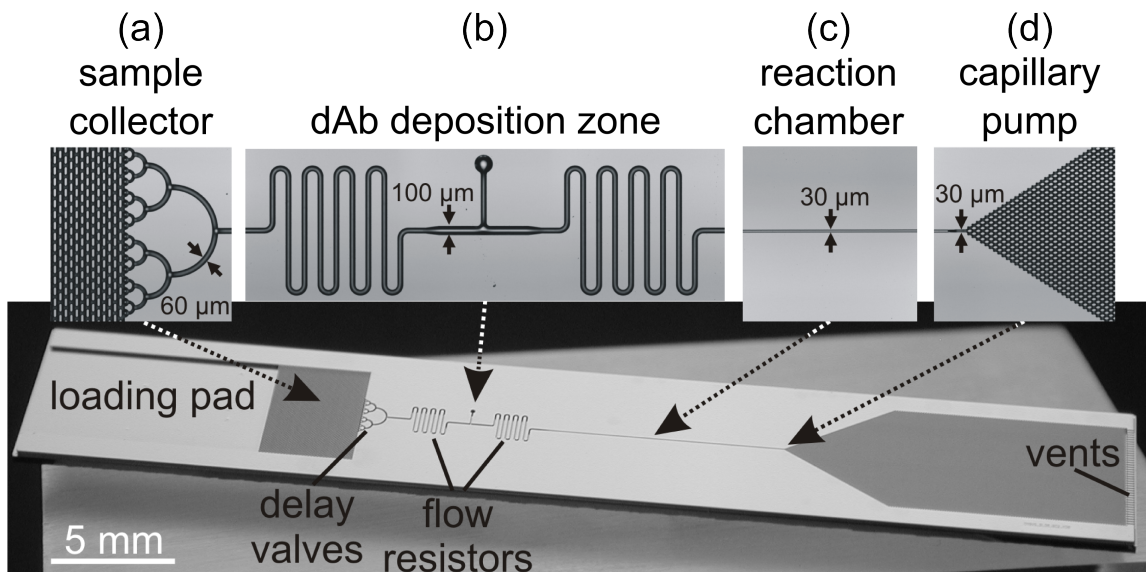


Figure 2.2: Optical photograph of the microfabricated Si microfluidic chip for one-step immunoassays having as main functional elements: (a) sample collector ending with hierarchical delay valves, (b) flow resistors and central deposition zone for dAbs, (c) reaction chamber and (d) capillary pump. All microfluidic elements are $180\ \mu\text{m}$ deep except for the reaction chamber that is $20\ \mu\text{m}$ deep. The chip is $43 \times 11\ \text{mm}^2$ and has an average flow rate of filling with human serum of $82\ \text{nL min}^{-1}$.

hours when the chip was exposed to ambient conditions. We therefore decided to use this method of treatment over coating the Si chip with gold that was subsequently derivatized with thiolated polyethylene glycol. [222]

The first microfluidic structure in the chip is the sample collector shown in Fig. 2.2a. It is $180\ \mu\text{m}$ deep and has elongated posts arrayed in a centered rectangular lattice. The vertical and horizontal distances between posts from edge to edge are $30\ \mu\text{m}$ and the capacity of the sample collector is $2\ \mu\text{L}$. The capillary pressure exerted on a liquid in the sample collector ranges from $-1.33\ \text{kPa}$ to $-2.94\ \text{kPa}$ between posts and is very good at wicking the liquid out of the Vivid[®] plasma separation membrane, composed of microstructured asymmetric polysulfone, down into the sample collector. The sample does not always reach the end of the sample collector with a straight filling front. For this reason, streams of sample are collected using delay valves. [159] These valves start with two inlets merging into one outlet and consolidate incoming streams of sample into one. A meniscus progressing through one inlet of a delay valve stops until it can merge with the meniscus of an adjacent

inlet. The menisci of the filling fronts move from regions of high capillary pressure (30 μm wide channels) into regions of reduced capillary pressure (45 μm and 60 μm wide channels). The channel width is gradually increased at each level to keep friction low and the flow rate similar. For example, from 5 levels of delay valves we can collect liquid from a 1900 μm filling front into a 60 μm wide channel without generating air bubbles. Once in the sample collector, the sample (here serum) needs only a few seconds to reach the delay valves and merge into a 60 μm wide channel.

The next set of microfluidic structures are flow resistors and a zone for depositing dAbs shown in Fig. 2.2b. The key concept to implementing a one-step immunoassay is to have all reagents on-chip. Solutions of dAbs are deposited onto the chip in a zone that is 180 μm deep and 100 μm wide using an inkjet that generates drops with a diameter of 70 μm . The deposition zone defines the redissolution profile of the dAbs when the assay is performed. Flow resistors are meandering microchannels that can be used to store overflow quantities of dAbs deposited in the deposition zone and prevent them from reaching the serum collector or the reaction chamber. The width, length and repeat number of curves can be varied to change the hydraulic resistance of the flow resistors. This can be used to change the redissolution profile of dAbs and the reaction kinetics of immunoassays that target different reagents or sensitivity levels. The flow resistors are 180 μm deep and 60 μm wide and have a capillary pressure of -1.92 kPa. The flow resistors and deposition zone have a total volume of 300 nL. The flow resistor is a convenient feature which can be changed at will to modify the hydraulic resistance of the entire microfluidic chip. This prevents having to redesign other more complex parts of the chip if chips requiring different flow rate characteristics must be fabricated.

The reaction chamber is shown in Fig. 2.2c. A PDMS substrate, patterned with lines of cAbs and lines of analyte, is placed onto the reaction chamber so that the lines are perpendicular to the main axis of the reaction chamber. The detection signal and positive control can be measured through the PDMS substrate in the reaction chamber using a fluorescent microscope. The redissolution of dAbs can also be observed in this way. The reaction chamber is 30 μm wide and 20 μm deep with a capillary pressure of -6.22 kPa and a total volume of 50 nL. The reaction chamber is shallower than the other parts of the

chip for accelerating the immunoassay reaction by providing short diffusion distances for the analyte-dAb complex to bind to the cAbs on the PDMS substrate.

The capillary pump shown in Fig. 2.2d contains rounded hexagonal posts arrayed in a centered rectangular lattice with a depth of $180\ \mu\text{m}$. The rounded hexagonal posts are used to define a reproducible filling behaviour that minimizes the formation of air bubbles. [180] The distance between hexagonal posts is between $15\ \mu\text{m}$ and $40\ \mu\text{m}$ and the capillary pressure accordingly varies from $-2.80\ \text{kPa}$ to $-7.20\ \text{kPa}$. The total volume of the pump is $10\ \mu\text{L}$. The capillary pump adds only a small flow resistance because it is composed of many parallel flow paths. Within a minute after the addition of the sample onto the loading pad, the sample fills the chip up to the capillary pump. The capillary pump has the largest capillary pressure on the chip and it determines the flow rate together with the flow resistor, and the maximum volume of sample used in the immunoassay. The area of the pump that was filled in a given time interval determined the average flow rate, which was in the case of human serum $82\ \text{nL min}^{-1}$ during a 14 minute one-step assay.

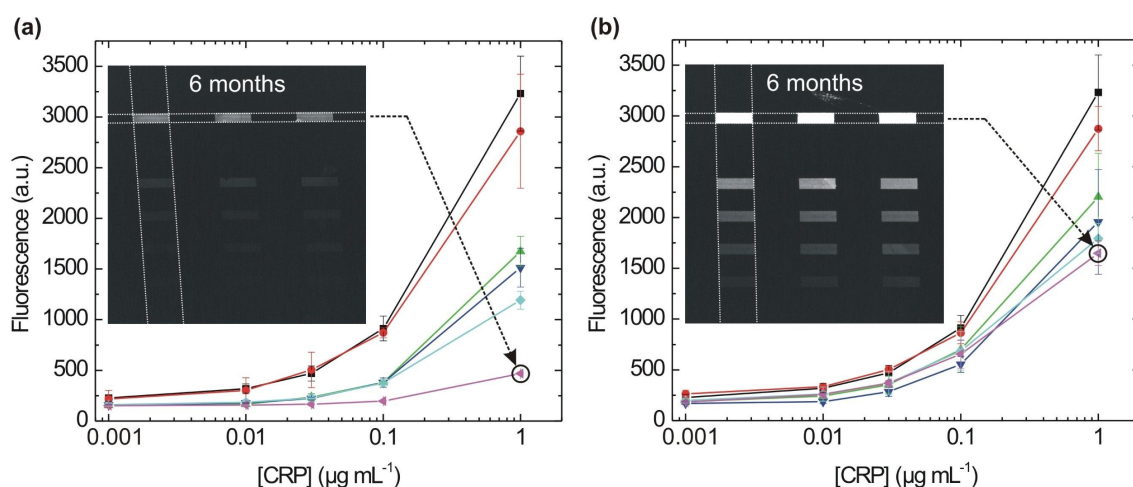


Figure 2.3: Study of the lifetime of cAbs on PDMS when stored at (a) room temperature and (b) room temperature with desiccant. Immunoassays were performed with human serum spiked with CRP and a capillary microfluidic chip that had 6 parallel reaction chambers (horizontal direction). The PDMS samples that had patterned cAbs (vertical direction) were (black square) fresh or stored for (red circle) 1 week, (green triangle) 2 weeks, (blue triangle down) 1 month, (turquoise diamond) 3 months, and (purple triangle left) 6 months. The fluorescent micrograph shows the strength of the fluorescent signal of PDMS substrates stored for six months (see text for details).

PDMS does not seem to be commonly used for disposable labware in biology although it is frequently employed in research. This is somewhat surprising because first, PDMS exhibits the lowest autofluorescence across the visible spectrum of many plastic and glass materials commonly used for biological assays. [42] Second, PDMS is chemically stable and highly transparent. Third, it is hydrophobic and therefore is easy to coat with proteins by simple adsorption from solution. Fourth, immunoassays performed on PDMS substrates can achieve picomolar sensitivity. [32] An essential question is whether it is suited for use in biological assays after being patterned with proteins and stored for large periods of time. The lifetime of cAbs on PDMS is probed by performing fluorescence immunoassays using human serum spiked to different concentrations of CRP: $1 \mu\text{g mL}^{-1}$, CRP free serum, 100 ng mL^{-1} , 30 ng mL^{-1} , 10 ng mL^{-1} , and 1 ng mL^{-1} , one concentration per horizontal reaction chamber from top to bottom, Fig. 2.3. The PDMS substrates were patterned with lines of cAbs perpendicular to the reaction chambers and stored at room temperature without and with desiccant for up to 6 months. The micrographs in Fig. 2.3a and b show the measured fluorescent signals corresponding to the different concentrations of CRP in the serum for either storage condition. An important loss of signal is observed after only one week when no desiccant was used, Fig. 2.3a. After 6 months, the signal measured for a concentration of CRP of $1 \mu\text{g mL}^{-1}$ falls to approximately 14% of the signal measured on PDMS freshly patterned with cAbs. This contrasts markedly with results obtained when the patterned PDMS substrates were stored with desiccant. In the latter case, the signal measured on 6 month old substrates corresponds to 51% of the signal measured on fresh substrates for a CRP concentration of $1 \mu\text{g mL}^{-1}$. The fluorescence microscope image in Fig. 2.3b even reveals that signals down to a concentration of 10 ng mL^{-1} are easily detected.

These results are important as they clearly indicate that PDMS can be used a long time after it has been patterned with cAbs and that similarly to proteins immobilized on other plastics (e.g. polystyrene) cAbs have a long lifetime when stored under dry conditions. Here, the high mobility of siloxane chains at room temperature and the presence of non crosslinked and/or low molecular weight species in PDMS do not seem to interfere with the biofunctionality of the layer of cAbs. Dry conditions favour the dehydration of proteins

and limit molecular mobility and rearrangements of their constituents thereby making (oxidative) degradation of proteins less likely. We suggest in addition that the lifetime could even be further augmented by adding stabilizing agents in the solution pipetted in the stencils. These agents typically have multiple hydroxyl groups that stabilize hydrophilic amino acids on protein shells. [227]

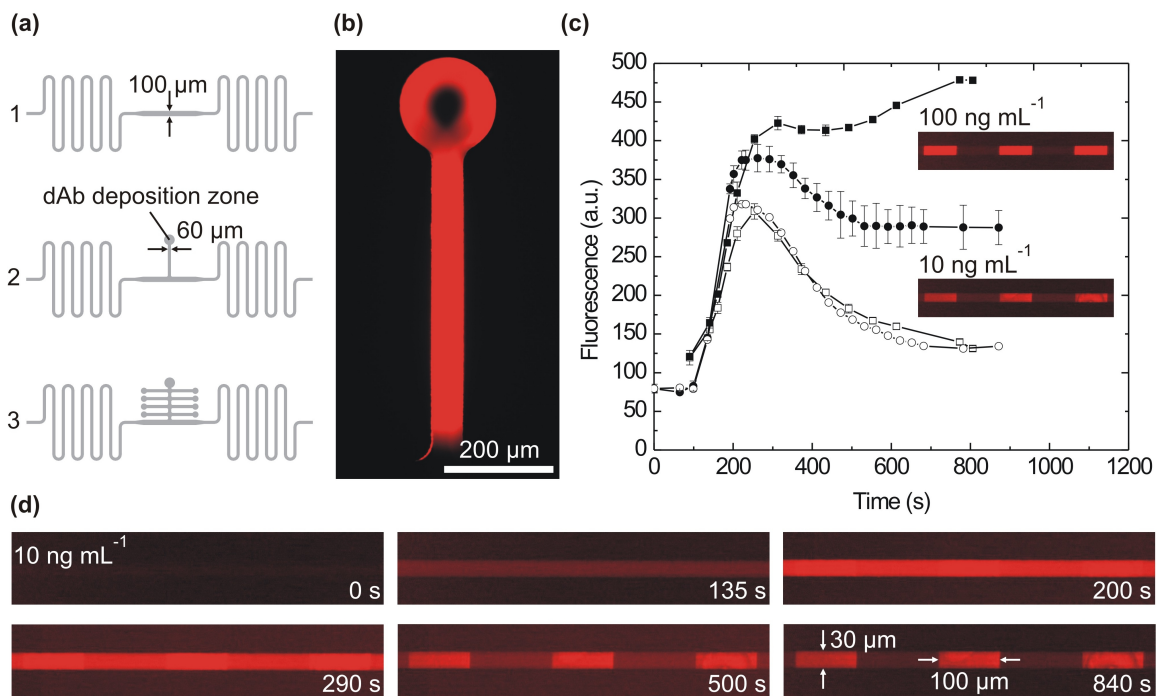


Figure 2.4: Deposition of dAbs on the microfluidic chip and its influence on the immunoassay. (a) The 3 layouts show examples of zones for depositing solutions of dAbs. This zone can be designed so as to vary the redissolution profile of dAbs and the vicinal flow resistors can be adjusted to define the flow rate in the microfluidic chip. (b) This fluorescence micrograph reveals the distribution of Alexa 647-labelled solution of dAbs, which was de-positied in a zone as in a2 using an inkjet (20 drops \times 3.6 nL) and dried. Such a deposition zone was used throughout the chapter. (c) The fluorescent signal measured on (specific signal) and in between (background signal) capture areas varies over time with the capture of analyte-dAb complex and the flow of dAbs in solution. The fluorescent intensity of capture areas is shown for (full square) 100 ng mL⁻¹ and (full circle) 10 ng mL⁻¹ and the corresponding background signal outside of capture areas for (empty square) 100 ng mL⁻¹ and (empty circle) 10 ng mL⁻¹. The shown fluorescence micrographs were taken approximately 14 min after adding the sample on the chip. (d) Fluorescence time lapse micrographs of 3 capture areas showing the increasingly visible detection of CRP from human serum that was spiked to 10 ng mL⁻¹.

The key to one-step immunoassays is having all reagents for the immunoassay on-chip.

In previous work, we pipetted by hand "large" volumes of dAbs of 150 to 500 nL on chips and shock froze them. [324] Here the deposition of dAbs is greatly simplified. Volumes of dAbs 40 times smaller are used and the deposition zone of the chip can be kept small compared to the rest of the chip owing to the precise spotting of solutions of dAbs using an inkjet. Deposition zones for dAbs determine the volume of dAbs that can be deposited and their redissolution profile when the immunoassay is performed. Three deposition zones for dAbs were tested, Fig. 2.4a. The first deposition zone was the simplest and consisted of a slightly enlarged channel between a set of flow resistors. Solutions of 3.6 nL of dAbs deposited in this deposition zone using an inkjet redissolve as a plug that completely passes through the reaction chamber within one minute. A sensitivity for CRP of 100 ng mL^{-1} was obtained with-in 2 minutes after sample introduction onto the loading pad. This deposition zone can be used for immunoassays of higher concentration samples that require quick time to results within a few minutes. In order to increase the sensitivity, the sample should preferably redissolve the dAbs into a larger plug to allow more time for forming the analyte-dAb complex and capturing them. The second deposition zone consists of a side channel ending with a circular well where 3.6 nL (20 drops) of a solution of dAbs was deposited. The circular well had a diameter of $200 \mu\text{m}$ to provide a large enough area for depositing $70\text{-}\mu\text{m}$ -diameter drops of dAbs using an inkjet microdispenser. When the sample fills the chip, it enters the side channel and progressively fills it thereby redissolving the dAbs. The liquid slows down at the edge of the circular well due to the entrapment of air there. This results in a depletion of the deposited dAbs that dissolve in the liquid, diffuse passively from the side channel to the main channel and are transported by the sample flowing toward the reaction chamber. Analyzing the intensity profile of the fluorescence in between the capture areas over time, we found that 90% of the dAbs dissolved in a sample volume of 850 nL. This geometry led to a sensitivity below 1 ng mL^{-1} and was employed throughout the rest of the chapter. The third deposition zone adds horizontal channels for depositing larger volumes of dAbs and for enabling longer incubation times. Sample slowly fills one horizontal channel at a time, entrapping air at each circular well. Then a large air bubble is formed in the entire deposition zone that propagates and blocks the main channel, creating a stop valve. When enough air permeates through the PDMS, after more than 40 minutes,

the main channel is unblocked and the sample plug reaches the reaction chamber. It was not possible to measure CRP at a concentration of 1 ng mL^{-1} within 30 minutes using the third deposition zone. Reducing the number of horizontal channels in a future design might be used to entrap less air and reduce the time required for air to permeate through the PDMS. In this way, higher sensitivities could be achieved with a programmed incubation time of the analytes with dAbs. [181]

Inkjet printers are very precise and can be aimed within small regions of the chip as shown in Fig. 2.4b. The total concentration of protein had to be approximately a maximum of 1 mg mL^{-1} in order to minimize viscosity and allow for the reproducible formation of droplets using the inkjet. The concentration of dAbs was $250 \text{ } \mu\text{g mL}^{-1}$ and L-phenylalanine was $700 \text{ } \mu\text{g mL}^{-1}$. L-phenylalanine was used to increase the total concentration of proteins in the dAb solution because proteins are more stable at higher concentrations. Additionally, L-phenylalanine creates a scaffold when dried that helps immobilize the dAbs and prevent denaturation. Trehalose and other sugars such as sucrose and glucose, are often used in protein solutions that are inkjet deposited and dried. These sugars help stabilize the proteins and slow down denaturation. Leaving the chip to dry in air after deposition using the inkjet was sufficient to provide a good particle free redissolution provided the trehalose concentration was high enough. There was no need for freeze-drying. Solutions of dAbs with a trehalose concentration of 25 mM redissolved with many particles and protein aggregates. The concentration was increased to 50 mM , 100 mM and 200 mM with the latter leading to the complete suppression of particles in the redissolution and thus it was used throughout the experiments. The microfluidic chip fabrication, inkjet deposition of dAbs, cAb patterning on PDMS and assembly into a device would be manufactured, shipped to the hospital and stored before use.

The evolution of the fluorescent signals measured through the PDMS over the reaction chamber for two separate chips after the addition of a sample with a concentration of CRP of 10 ng mL^{-1} and 100 ng mL^{-1} is shown in Fig. 2.4c. The curves obtained from the background signals measured between the capture areas are very similar for both chips. The deposition and redissolution of dAbs between two separate chips is reproducible. The specific signals measured on the rectangular capture areas contain the signal from

analyte-dAb complexes on the cAbs and the background signal of nearby dAbs in solution. Micrographs taken from the timelapse video of the detection of a concentration of CRP of 10 ng mL^{-1} are shown in Fig. 2.4d. The dAbs are gradually redissolved and pass through the reaction chamber to reach a peak in background signal at 250 s after which the number of dAbs in the sample gradually decreases and the specific signal becomes increasingly visible. The capture areas are homogenous and small with dimensions of $30 \mu\text{m}$ by $100 \mu\text{m}$. Three capture areas occupy only a length of $600 \mu\text{m}$. These capture areas can be patterned with up to 16 different cAbs to provide multiplexed detection of several analytes and with different patterning there is no need to use different fluorescent markers. In addition, the dAb deposition zone is large enough to accommodate larger amounts of dAbs without redesigning the chip. A supplementary video shows the evolution of the fluorescent signal from dAbs in the reaction chamber and the increase of the signal on the PDMS substrate after the addition on the chip of human serum spiked with CRP at a concentration of 1 ng mL^{-1} .

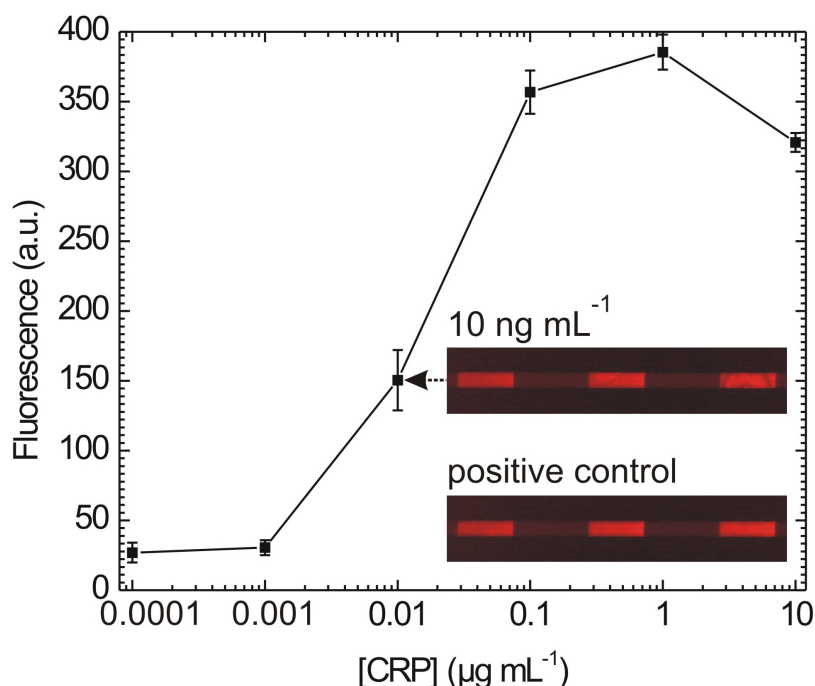


Figure 2.5: Graph of the fluorescent signal obtained for immunoassays done with different CRP concentrations on the one-step chip. The fluorescence micrographs respectively correspond to the signal and positive control of detection of CRP from human serum that was spiked to 10 ng mL^{-1} obtained 14 min after adding the sample on the chip.

The graph shown in Fig. 2.5 was plotted from the detection signal obtained on separate one-step chips 14 minutes after the addition of sample. The sensitivity was at least 1 ng mL^{-1} . The micrographs show a clear specific signal for a concentration of CRP of 10 ng mL^{-1} and a positive control signal on the same chip where dAbs bind to CRP on the PDMS surface. Above $1 \text{ } \mu\text{g mL}^{-1}$ CRP the high-dose hook effect is observed. [325] The excess analyte saturates both the dAbs and cAbs and prevents to some extent the analyte-dAb complex from binding to the cAbs on the PDMS substrate. This commonly happens in solid phase assays when there are limited quantities of dAbs. Adding more dAbs would lead to a flattening of the curve at high concentrations. In healthy adults, normal CRP concentration can range from $0.8 \text{ } \mu\text{g mL}^{-1}$ to $10 \text{ } \mu\text{g mL}^{-1}$, and following an acute-phase stimulus, CRP concentration can increase to more than $500 \text{ } \mu\text{g mL}^{-1}$. [326] To target this concentration range, the sensitivity of the chips can be adjusted to detect higher concentrations of CRP, for example by using antibody pairs with less affinity to CRP, or by varying the flow rate of sample in the reaction chamber.

Implementing positive control lines after the capture areas provides two benefits. First, a user can verify that the sample has filled the reaction chamber and passed the capture areas up to the capillary pump indicating that the chip has filled properly. Second, the stability, activity and redissolution of dAbs can be measured and test results can be interpreted as non-valid or valid based on the normality of the positive control signal. A doctor or nurse may use the device by adding $5 \text{ } \mu\text{L}$ of patient sample (blood or serum), waiting 15 minutes and inserting the device into a fluorescence reader. The device would be ideal for clinicians who tend to observe the evolution of CRP levels in the blood of patients to perform prognostics: for example, an increase in concentration of CRP in a patient's serum can indicate a worsening of sepsis [327] or a diminution can indicate a recovery after an acute myocardial infarction. [328, 329]

2.5 Conclusion

The one-step chip presented here provides many features. The chip is autonomous and requires only the addition of a drop of sample. The sample is merged seamlessly from several parallel filling fronts into a single channel by delay valves without forming air bubbles. Nanoliter volumes of dAbs are incorporated on-chip and redissolved without particles. Sixteen lines of cAbs and analytes for positive control are patterned onto a PDMS cover. CRP is detected at a concentration below 1 ng mL^{-1} in 5 minutes using $5 \text{ }\mu\text{L}$ of human serum. The use of a peripheral fluorescence reader keeps active components outside of the chip and provides sensitive concentration measurements of analytes for patients' digital health record that should help doctors use test results in standard operating procedures and administer the appropriate treatment. The main task that remains is to fabricate the chip using plastic components to create a disposable chip for use in hospitals and elsewhere. This should however not be difficult considering the capabilities of plastic engineering using injection molding or hot em-bossing.

3

Multiparametric microfluidic chips for one-step immunoassays

3.1 Preface

Here we present a capillary-driven microfluidic chip for “one-step” immunoassays. The chip allows for easy modification of several assay parameters such as the flow rates of sample, the volumes of samples for tests, and the type of reagents and receptors for analytes. We therefore term such a chip a multiparametric chip and illustrate this concept with the integration and release of anti-CRP dAbs together with splitting flow of samples containing CRP across lines of anti-CRP cAbs. The microfluidic chip is fabricated in Si and is sealed with PDMS patterned with cAbs. The microfluidic chip is $\sim 1.7 \times 3.4 \text{ cm}^2$ and is capable of analyzing $20 \mu\text{L}$ of human serum in 6 parallel flow paths with a range of flow rates from 3.3 nL s^{-1} to 0.46 nL s^{-1} . An inkjet spotter was used to deposit 10.6 nL of dAb solution in a structure vicinal to the main flow path of the chip. The consequent asymmetric release of dAbs in a stream of human serum is compensated by a Dean flow mixer having 9 mixing loops and a footprint of $\sim 2.8 \times 0.78 \text{ mm}^2$. The quantity of dAb present in the half of the flow path close to the spotting region decreases from 83% at the entrance of the mixer to 52% in the region after the mixer. The sample is then equally split into 6 reaction chambers

and proceeds via connecting channels to 2 μL capillary pumps. The hydraulic resistance of the connecting channels is designed to vary flow rates, and therefore the kinetics of capture of CRP-dAb complexes, from 10 minutes to 72 minutes. The increased incubation time leads to a fourfold increase in detection signal in the reaction chamber with the longer incubation time. The concept presented here is flexible and suited for implementing various surface fluorescence immunoassays on a capillary-driven microfluidic chip. This chapter is based on Capillary-driven multiparametric microfluidic chips for one-step immunoassays, L. Gervais, M. Hitzbleck, E. Delamarche, *Biosensors and Bioelectronics*, 2011.

3.2 Introduction

Immunoassays are central to research in the life sciences and clinical diagnostics; [330] they are routinely performed to detect disease markers and pathogens in patients as well as for therapy monitoring. [1] Portable devices for immunoassays are increasingly used in healthcare for decentralized and point-of-care testing, [331] providing benefits of a shorter time to result and accessibility where there is limited infrastructure.

Originally, lateral flow assays on porous membranes were developed to provide a portable format for immunoassays. [332] The immunoassay strip test, such as the one used in a home pregnancy test, was the first commercially successful example of point-of-care immunodiagnostics. Recent developments of this technology have focused mostly on improving biochemical receptors (antibody fragments, aptamers, engineered proteins, etc.), detection labels (nanoparticles, quantum dots, fluorescent dyes, etc.) and peripheral detection systems (readers, light emitting diodes, lasers, CCD chips, etc.). Flow paths for immunoassays allowing routing and mixing have been made of paper, [36] thread, [53] and polymers. [40] One aspect of these approaches is the increasing challenges of scaling down the volumes of sample that are needed, miniaturizing signals for multiplexed analysis, accurately controlling flow conditions for precise reaction protocols, and the difficulty of providing a range of quantifiable detection signal intensities.

Microfluidic technology has the opportunity to extend the capabilities of lateral flow assays. For example, one commercially successful test performs one-step immunoassays for the detection of three cardiac markers. [30, 320] Here, further miniaturization and

multiplexing is challenging due to the large footprint required for detection zones and because all reagents are integrated into one channel leading to crosstalk when the number of reagents is increased. Liquids can be passively driven in microfluidic immunoassay devices using several approaches such as capillary forces that are generated by microstructures, [98, 183] gravity driven flow in tilted microchannels [186] and liquid suction from degassed PDMS dissolving air from microchannels. [181] In particular, capillary driven microfluidics were used for one-step immunoassays from human serum [31] and immunoassays using multiple successive pipetting steps for the detection of eleven markers from blood. [199]

Despite important efforts to implement lateral flow assays on microfluidic formats, major limitations remain. For example, flow paths tend to be designed in a “linear” manner where areas containing reagents for a test are placed one after another. Reaction conditions are also preset or necessitate peripheral equipment to activate valves, release reagents, and achieve flexibility in flow rates. [110, 185] Different markers usually require specific reaction conditions for optimal detection because of the range of clinically relevant concentrations and the variability in binding characteristics between analytes and receptors. Additionally, the variability of clinical samples, such as the difference in viscosity between samples from different patients, and changes in ambient conditions (temperature and humidity) when the test is done can challenge tests that have preset parameters. For these reasons, it is difficult to implement the detection of a panel of markers on a passive device.

Here, we present one-step microfluidic chips for immunoassays that have split flow paths that can impose different flow rate conditions on samples added to the chips. The flow of sample in these chips is governed by capillary forces. Reagents are integrated in the chips and are released in a stream of sample so as to enable immunoreactions on miniaturized capture areas. This addresses the above mentioned limitations and builds on our earlier work on one-step immunoassays. Additional goals of this work are to keep the chip compact, simple to use, and working with the principles of one-step immunoassays as well as to provide a solution that can be low cost to manufacture, both by preventing the integration of active components and active packaging methods and by using designs that can be transferred from Si technology to plastic replication.

3.3 Experimental

3.3.1 Chemicals, biochemicals and PDMS

Water was deionized with a Simplicity 185 system (Millipore, Billerica, MA). PBS was prepared by dissolving PBS tablets (Sigma-Aldrich, St. Louis, MO) in deionized water and the resulting solution was filtered using a 0.20 μm syringe filter (Sartorius, Epsom, U.K.). Solution of 1% w/v BSA (Sigma-Aldrich) was prepared in PBS. Ethanol (puriss $\geq 99.8\%$, Fluka, Sigma-Aldrich) was used to rinse Si chips and PDMS substrates. PDMS layers were prepared by mixing Sylgard 184 pre-polymers (Dow Corning, Midland, MI) at a ratio of curing agent to polymer of 1:10 using a DOPAG mixer (Cham, Switzerland). The mixture was poured onto planar polystyrene Petri dishes (Greiner BioOne), and cured overnight at 60°C in an oven. The approximately 1-mm-thick cured PDMS was cut using a scalpel into pieces having approximately the size of a microfluidic chip and patterned with cAbs as previously published. [222] Samples for immunoassays were prepared using human CRP-free serum (8CFS, HyTest, Turku, Finland) that was spiked with CRP (8C72, HyTest). Anti-CRP-C2 Abs (4C28-C2, HyTest) were used as cAbs. Anti-CRP-C6 (4C28-C6, HyTest) were used as dABs. These Abs were labeled with Alexa Fluor 647 (Invitrogen, Carlsbad, CA) following the instructions from the supplier. Each dAbs had approximately 4 fluorophores as measured using an Eppendorf BioPhotometer (Hamburg, Germany). A solution of 1% w/v Pluronic® F108 (BASF, Ludwigshafen, Germany) in deionized water was used to treat the Si chips. A solution of 2% v/v Hellmanex II (Hellma, Mullheim, Germany) in deionized water was occasionally used to clean the inkjet glass pipettes. Solutions of dAbs were prepared using 250 $\mu\text{g mL}^{-1}$ Alexa 647 labeled anti-CRP-C6, 68 mg mL^{-1} D-(+)-trehalose (Fluka) and 700 $\mu\text{g mL}^{-1}$ L-phenylalanine (Fluka) in PBS. Solutions of cAbs were prepared in PBS at a concentration of 125 $\mu\text{g mL}^{-1}$.

3.3.2 Fabrication of chips and preparation

Photolithography, quartz chrome masks (Photronix, Dresden, Germany) and 4-inch Si wafers (Siltronix, Geneva, Switzerland) were used to fabricate the microfluidic chips and stencils. For the microfluidic chips, a 0.5 μm thick layer of SiO_2 was grown onto

a Si wafer using wet oxidation in a thermal furnace (TS-6304, Tempres, Vaasen, The Netherlands). Hexamethyldisilazane (adhesion promoter) was first spin coated onto the wafer. Then photoresist (AZ 6612, MicroChemicals, Ulm, Germany) was spin coated at 4000 rpm to a thickness of approximately $1.6\ \mu\text{m}$ and soft baked for 1 min at 110°C . A two-layer-mask process was used. The first layer contained all the microfluidic structures of the microfluidic chip. The second layer contained all the features except the reaction chambers. The first layer mask was brought to a “soft” contact with the resist-coated wafer and exposed to $6.5\ \text{mW cm}^{-2}$ ultraviolet light for 15 s using a mask aligner (MA/BA6, Süss Microtech, Garching, Germany) and developed. Buffered hydrofluoric acid (BHF, 7:1 volume ratio of 87.5% NH_4F in water to 12.5% HF in water; danger) was used to etch SiO_2 from the microchannels uncovered by the photoresist. The resist was stripped from the wafers and HMDS was spin coated. Photoresist (AZ 4562, MicroChemicals, Ulm, Germany) was spin coated at 4000 rpm to a thickness of approximately $7\ \mu\text{m}$ and soft baked for 1 min at 110°C . The second layer mask was exposed to ultraviolet light for approximately 45 s and developed. The second layer microchannels were etched into Si to a depth of $160\ \mu\text{m}$ using deep reactive ion etching (AMS-200SE, Alcatel Micro Machining Systems, Annecy Cedex, France). The resist was stripped from the wafer and the first layer was etched to a depth of $20\ \mu\text{m}$ using reactive ion etching. The fabricated microfluidic chip was composed of $180\ \mu\text{m}$ deep microchannels and $20\ \mu\text{m}$ deep reaction chambers.

Stencils consisted of $12 \times 12\ \text{mm}^2$ chips containing 4 groups of 4 trenches. Each trench was etched through the wafer and comprised a loading pad (for pipetting a solution) connected to a 2-mm long and $100\text{-}\mu\text{m}$ -wide channel. A one mask photolithography process was used to pattern resist and transfer the resist pattern through the wafer using deep reactive ion etching to form the stencils. A pattern delineating the perimeter of each stencil was etched as well through the wafer, which resulted with the release of individual stencils.

Air-based plasma (Tepla microwave-plasma system 100, PVA Tepla, Asslar, Germany) at 200 W and 0.7 Torr was used to clean the microfluidic chips for 2 min. The microfluidic chips were then incubated in a solution of Pluronic[®] for 30 min, rinsed with deionized water and dried under an N_2 stream. Microfluidic chips were stored in a dark and dry

environment and used within 4 hours of coating with Pluronic®.

3.3.3 Capture antibody patterning on PDMS

Air-based plasma was used to clean the stencils and make them hydrophilic. Single side polished Si wafers were used to fabricate the stencils. The polished side of the stencils was placed on top of the Petri-touching side of the PDMS substrates. Capture antibodies were deposited on PDMS at room temperature by pipetting a solution of cAb in the stencils and incubating for 15 min. A small Petri dish with sheets of clean room paper soaked in water at the bottom was used as a humidity chamber to store the chips during incubation and prevent evaporation of the solution of cAb in the stencil. Stencils were rinsed with PBS (~10 mL) and deionized water (~10 mL) before removing them from the PDMS substrates using tweezers under a bath of blocking solution of BSA. The PDMS substrates were left in the solution of BSA for 15 min before rinsing them with PBS and deionized water, and drying under a stream of nitrogen. Stencils were rinsed in deionized water, ethanol and cleaned with an air-based plasma before they were reused.

3.3.4 Immunoassays

An Autodrop MD-P-705-L (Microdrop, Norderstedt, Germany) inkjet printer and an AD-K-501 piezo-driven pipette having a nozzle of 70- μm in diameter were used to deposit a solutions of dAbs in the microfluidic chips. The inkjet pipette nozzle was aligned with the detection antibody dissolution zone and 60 drops of dAb solution of 180 pL each were deposited for a total of 10.8 nL. Microfluidic chips were stored in the dark and used within 4 hours of deposition.

A PDMS substrate was placed on the microfluidic chip in such a way that perpendicular lines of cAbs crossed the reaction chambers of the chip and that the chip was covered from the pumps up to half of the loading pad. The microfluidic chip was placed on the stage of a fluorescence microscope. Immunoassays started when 20 μL of human serum spiked with CRP at 100 ng mL⁻¹ was pipetted into the loading pad of the microfluidic chip. The fluorescence in the different chip regions was monitored directly through the PDMS cover. Fluorescence images of different parts of the microfluidic chip were captured at various

times during the experiment. Fluorescence images were captured along the Dean flow mixer and stitched together using an image processing software. The flow distributor was monitored throughout the experiment. After experiments, microfluidic chips were rinsed with deionized water, ethanol, and dried under a stream of N_2 . The microfluidic chips were cleaned with an air-based plasma and ready for reuse.

3.3.5 Instrumental setup and data acquisition

Fluorescence measurements were done using a fluorescence microscope (Eclipse 90i, Nikon, Japan) and images were typically acquired using a $10\times$ objective. The microscope had a 100 W mercury arc lamp and a black and white CCD camera (DS-1QM, Nikon) cooled to -30°C . Fluorescence images were analyzed using NIS-Elements software (Nikon).

3.4 Results and Discussion

3.4.1 Chip design and filling

The selected strategy to vary assay conditions uses parallel flow paths on a microfluidic chip, Figure 3.1a. The sample initially follows a common flow path that includes a loading pad, a dAb dissolution zone and a Dean flow mixer and then splits into six individual reaction chambers. Six connecting channels join the end of each reaction chamber (at an area denoted J in Figure 3.1a) to an individual capillary pump. These connecting channels have dissimilar depths and lengths and therefore modulate flow rates throughout the 6 parallel flow paths via their specific flow resistance characteristics, Figure 3.1b.

A test begins when a sample is pipetted onto the loading pad, Figure 3.1c. The dAb dissolution zone is a side channel having a circular reservoir of branching channels that accommodate dissolved dAbs spotted using an inkjet printer and dried. The dAbs redissolve into one side of the central channel mostly by passive diffusion. The mixer distributes the concentration of dAbs across the channel before splitting into six reaction chambers. The analyte-dAb complex binds to lines of cAbs on PDMS covering the reaction chamber. With all the reaction chambers seeing the same concentration of analytes and

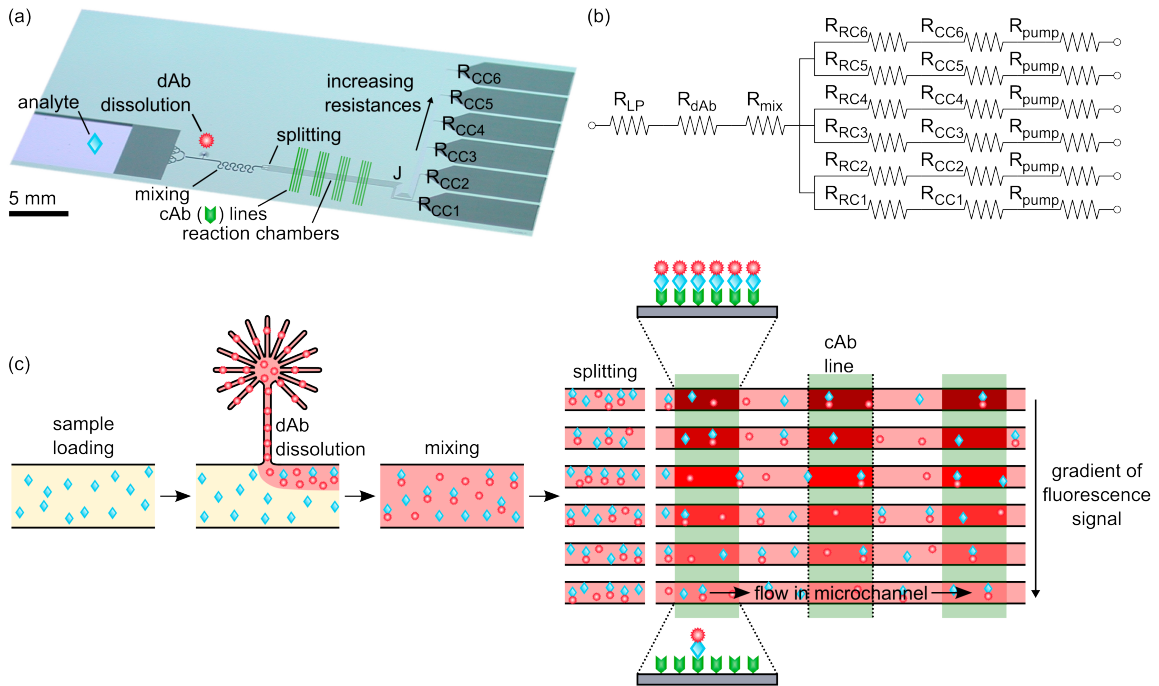


Figure 3.1: Capillary-driven microfluidic chip for multiparametric immunoassays. (a) Photograph of a chip having regions for holding and dissolving dAbs, mixing them in a stream of sample and splitting the sample into 6 streams passing over cAb areas at flow rate conditions determined by the resistance of channels connecting to individual capillary pumps. (b) Equivalent electrical model of the chip used to calculate flow rates and filling times. (c) Illustration of the release and even distribution by mixing of dAbs in a stream of sample passing through the different components of the microfluidic chip and that can result in variable amount of signal in the case of a sandwich fluorescence immunoassay as show here.

dAbs passing through, we expect to see a gradient of fluorescence detection signal as a result of having dissimilar flow rates in the reaction chambers. The detection signal should be the highest when flow rates are reduced leading to increased incubation times. Other parameters than the flow rate can be varied with the chip shown in Figure 3.1 such as, for example, the types of dAbs integrated in the chip and receptors patterned on PDMS.

The flow resistances, flow rates and filling times of the flow paths of the microfluidic chip shown in Figure 3.1 were calculated following prior work [333] and are summarized in Table 3.1. First, we calculated the capillary pressure of a single capillary pump. This was done using a value of 56.2 mN m^{-1} for the surface tension of the serum [334] and 20° and 116° for the respective contact angles of the serum on the walls of the microfluidic flow

paths and the PDMS. The pump is an array of microstructures with separation distances that generate two levels of capillary pressures (-2.8 and -7.2 kPa) depending on the position of the meniscus in the array. [159] The weakest pressure (-2.8 kPa) is the main factor limiting flow in the pumps and was used to calculate approximate flow rates of the individual flow paths. For this, the hydraulic resistance of each flow path between the loading pad and its corresponding pump was calculated by summing the contribution of each element composing the flow path. These resistances correspond to the loading pad (R_{LP}), the dAb dissolution zone (R_{dAb}), the Dean flow mixer (R_{mix}), one reaction chamber (R_{RC1-6}), a specific connection channel (R_{CC1-6}) and a capillary pump (R_{pump}). Taking a value of 1.8 mPa s [335] for the viscosity of the serum at 20°C, we estimated the flow rate to range from 1.1 to 6.3 nL s⁻¹ for the slowest and fastest flow paths, respectively. The corresponding time needed to fill the 2 µL volume of each capillary pump is consequently estimated to range from 8 to 47 min.

The resistance R_{CC1} was designed to have a filling time of the pump of approximately 10 min, which is suited for a fast diagnostic test. Shorter times were not considered here, although tests performed in just a few minutes might be beneficial in situations such as the diagnostics of heart disease and stroke. Additionally, the resistance of this connecting channel cannot be easily lowered without a major redesign of the microfluidic chip. For example, R_{CC1} was kept small by shifting all the structures (including the loading pad) before the connecting channels to one edge of the chip, and by shortening the first reaction chamber by 50% and keeping the depth of the first connection channel at 180 µm. In the eventuality that a diagnostic test of just a few minutes would be desirable, capillary pumps having a smaller volume can be used. The next resistances (R_{CC2-6}) were designed to increase the filling time incrementally to reach an upper limit of about 1 hour, which is a practical upper time limit for diagnostics tests. The experimental filling times, shown in Table 3.1, were measured by observing the time for a pump to fill completely. The experimental filling time of the first 4 flow paths are in line with the calculated ones and the last two are respectively 42% and 53% slower than the calculated ones. Possible reasons for this could be the difference between the viscosity and surface tension of human serum used for calculations and experiments. Some evaporation of sample through the PDMS covering

Table 3.1: Flow characteristics of the microfluidic chip.

Flow path	Total Resistance (m^{-3})	Flow rate D (L/s)	Calculated filling time	Experimental filling time
1 (R_{CC1})	3.8×10^{17}	6.3×10^{-9}	8 min 3 s	10 min
2 (R_{CC2})	7.5×10^{17}	3.2×10^{-9}	16 min 10 s	19 min
3 (R_{CC3})	1.5×10^{18}	1.6×10^{-9}	32 min 23 s	36 min
4 (R_{CC4})	1.8×10^{18}	1.4×10^{-9}	37 min 1 s	43 min
5 (R_{CC5})	2.0×10^{18}	1.2×10^{-9}	42 min 11 s	60 min
6 (R_{CC6})	2.2×10^{18}	1.1×10^{-9}	47 min 5 s	72 min

the microfluidic chip and in the loading pad may both increase the viscosity of the sample over time and compete with the filling of the pumps linked to the slowest flow path.

The microfluidic chip has a factor seven in filling time between the fastest and slowest flow paths, which can be used to shift the advancement of immunobinding reactions, [330] dissolution profiles of reagents, [336] and can also compensate for changes in sample characteristics. Filling time can be further increased if needed by routing resistances in the area left free in the chip. The viscosity of body fluid samples can vary greatly. For example, at 37°C in healthy adults, whole blood viscosity can be in a range of 2.83 to 6.3 mPa s [337] and whole saliva can be in a range 2.75 to 15.51 mPa s. [338] The viscosity of a sample is also affected by temperature. A whole blood sample can vary in viscosity in a range from 5.48 to 14.41 mPa s when temperature varies from 38°C to 5°C. [339] These viscosities can vary six fold between different samples and conditions. Since the flow rate is proportional to the viscosity of the sample, resistances in microchannels can be used to encode the same flow rates with samples of different viscosities.

3.4.2 Mixing

The dAb reagents enter the laminar stream of sample flowing along the lateral wall of the channel. They need to be evenly distributed across the channel before equal quantities of dAbs are split into 6 individual reaction chambers. If only passive diffusion is used, the dAbs, which are 150 kDa immunoglobulins G, would need a 2.87-cm-long channel to mix across a channel. The 125 kDa pentameric form of C-reactive protein, would need a 2.7-cm-long channel to mix across a channel. A channel this long would not only add

significant resistance but would also occupy significant area on the chip. Even for other analytes commonly detected using immunoassays, the channel length needed for mixing would be very long; 2.2-cm-long for serum albumin (64 kDa), and 9.7-mm-long for insulin (51 amino acids, 5.8 kDa), a small amino acid fragment and one of the smallest detectable molecules using immunoassays.

We therefore implemented a mixer on the chip to mix dAbs across the channel while using a smaller footprint than a straight channel. Many strategies have been developed for mixing in microfluidics. Active mixers are not considered here because they require external energy input, are complex and expensive to manufacture on microfluidic chips. Several passive mixers, requiring no external energy input and using simple geometries, can easily be integrated into diagnostic devices. For example, porous polymer embedded in microchannels can mix liquids in a short microchannel at the cost of increased complexity of fabrication. [38] Staggered groove structures in the floor of a microchannel can induce chaotic flows and mix liquids. [191] Mixers requiring only one lithography step for fabrication are simpler to manufacture and lower cost. For example, a mixer using modified Tesla structures can divide flow into two substreams that are merged and collide in opposite flow directions. [193]

Among passive mixers, we found the Dean flow mixer to be particularly attractive because it is simple to fabricate and composed of a geometry of looping channels that do not entrap air when filling with sample using capillary forces. The Dean flow mixer switches the sample between flow vortices of opposite direction that allow molecules to explore the channel cross section chaotically. [192, 340] The Dean number, which is a dimensionless number used to quantify the efficiency of mixing in curved channels, is

$$D_e = R_e \sqrt{\frac{d_H}{R}}$$

where R_e is the Reynold's number, d_H the hydraulic diameter and R the radius of curvature. The Reynold's number depends on the flow rate encoded in the microfluidic chip. The width and depth, with which the hydraulic diameter is calculated, were kept constant at 100 μm and 180 μm respectively, so that the Dean flow mixer could be integrated with the channels of the same dimension on the microfluidic chip. The radius of curvature was kept

as small as possible, $200\ \mu\text{m}$, in order to increase the Dean number. The calculated Dean flow number for the mixing loops in the microfluidic chip was 31, which is above 15 where enhanced mixing via chaotic flows begins. Moreover, the Dean flow mixer resistance of $1.0 \times 10^{15}\text{m}^{-3}$ contributes minimally to the overall resistance of the flow paths.

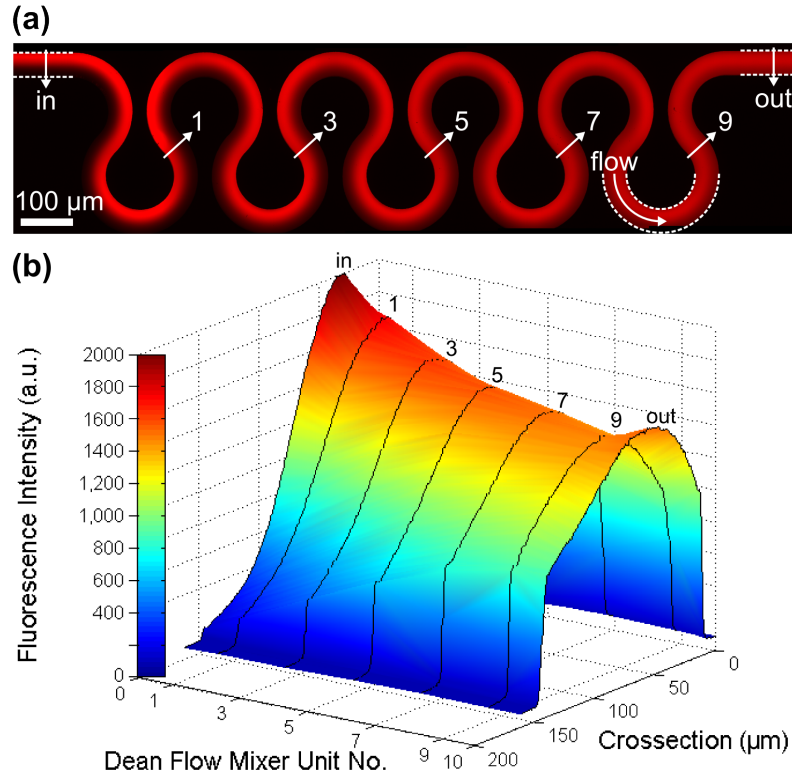


Figure 3.2: Fluorescence profile of sample containing fluorescently-labeled dAbs flowing through a Dean flow mixer. (a) Fluorescence image of the mixing of dAb solution diffusing into the lateral wall of the channel (top of the image) from the dAb dissolution zone and going through a series of mixer loops. The fluorescence intensity is measured from cross-sections of the microchannel denoted by the numbered arrows for the number of Dean flow loops. (b) Graph of the fluorescence intensity of cross sections showing concentrated dAbs along the lateral wall of the channel being gradually homogenized to the middle of the channel once they reach the end of the mixer.

Microscope fluorescence images of dAbs in sample flowing in an above-described Dean flow mixer are shown in Figure 3.2a. The images were captured 3 min after the sample was introduced into the loading pad, once the microchannels were filled and the capillary pumps determined the flow rate. The mixing effect is observed by looking at the signal cross section after every Dean flow loop, 3.2b, and quantified by measuring the portion of

dAbs that remain in the top half of the channel after diffusing from the dAb dissolution zone. At the entrance of the mixer (0th loop) there is a peak of 83% of dAbs in the top half of the channel. After the 9th loop, only 52% of dAbs remain in the top half of the channel. At the outlet, a bit more fluorescence is in the center of the channel. This could be due to fluorescent antibody agglomerates and antibody-antigen complexes being focused towards the middle of the channel by alternating Dean flow loops. [122]

3.4.3 Distribution and detection

Once the distribution of dAbs is improved by the Dean flow mixer, the sample and dAbs proceed to a region where the central channel is split into 6 reaction chambers where individual immunoassays are performed. The distribution channels, 3.3a, and the detection areas in the reaction chambers, 3.3b, were imaged at 30 min when flow paths 1 to 3 were filled, and flow paths 4 to 6 were still filling and contributing to the signal. The cross section intensity profile of the flow distributor, 3.3c, shows the same amount of dAbs flowing through each channel. The intensity profile of the signal areas, 3.3d, shows a four-fold increase in signal in the slow filling flow path 6 compared to the fast filling flow path 1.

Frequently, in the context of medical diagnostics, a few analytes occurring in different concentration ranges may be detected. Antibodies of different affinities can be used to vary the strength of the generated signal based on the desired concentration range of detection of analytes. Conversely, when keeping antibodies and antigens the same, the affinities cannot be changed, however the incubation time of analytes with receptor areas can be varied with the flow rate to vary the signal. An increased signal can be generated with increased incubation times due to slower flow rates that allow molecules more time to diffuse and interact with the detection areas. A decreased signal can be generated with decreased incubation times due to faster flow rates that do not allow molecules much time to diffuse to the surface. A microfluidic chip with multiple channels providing a range of signals for a given concentration of analyte, increases the detectable range of this analyte. This allows tuning for the optimal flow rate needed to detect an analyte antibody interaction of a given affinity. For example, using microfluidic chips with active pumping of sample, dAb solution, rinsing, and a flow rate 33 nL/min, a detectable signal for 10 pg mL⁻¹ of CRP

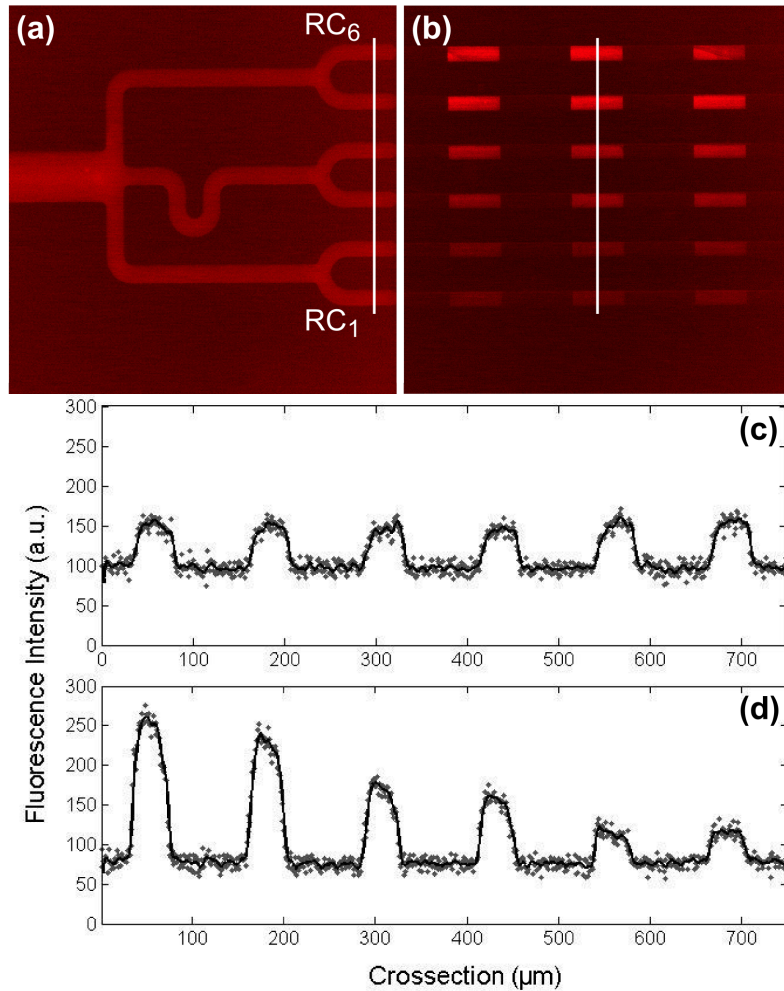


Figure 3.3: Fluorescence images of the distribution of fluorescently labeled dAbs (a) before and (b) at the sites with receptors and (c, d) corresponding intensity profiles (measured from top to bottom). (c) Intensity profile showing a similar quantity of dAbs entering RC_6 to RC_1 . (d) Intensity profile measured across the middle signal areas and revealing a fourfold increase in signal in RC_6 compared to RC_1 . Channels with slower flow rates, such as RC_6 , provide longer incubation times for analyte-dAb complexes to bind to cAb lines and increase signal compared to channels with faster flow rates and shorter incubation times, such as RC_1 .

in human serum was found (results not shown). In this way, low flow rates can be used to detect low concentrations of analytes.

Multiplexing could be implemented on a microfluidic chip similarly to micromosaic immunoassays. [341] On such a chip, a cocktail of dAbs would be inkjet deposited in the dAb dissolution zone. Six independent reaction chambers on which parallel lines of different cAbs can be patterned on the same chip would detect for clinically relevant markers in different concentration ranges.

3.5 Conclusion

A microfluidic chip for effecting multiparametric immunoassays was implemented. The microfluidic chip can accommodate various volumes of sample and dAb reagents, varying flow conditions and incubation times in 6 independent reaction chambers, and as a consequence can vary the detection signal strength based on incubation times, and has the possibility of multiplexing the detection of several analytes by inkjet depositing a cocktail of dAbs in the dAb dissolution zone and different lines of cAbs over the reaction chambers. We believe that such microfluidic chips will become increasingly attractive for decentralized diagnostics due to their flexibility, performance, simplicity of use and competitive low cost of fabrication when produced in polymeric materials.

4

Controlled release of reagents in microfluidics using reagent integrators

4.1 Preface

The integration and release of reagents in microfluidics as used for point-of-care testing is essential for an easy and accurate operation of these promising diagnostic devices. Here, we present microfluidic functional structures, which we call reagent integrators (RIs), for integrating and releasing small amounts of dried reagents (ng quantities and less) into microliters of sample in a capillary-driven microfluidic chip. Typically, a RI has an inlet splitting into a central reagent channel and two diluter channels. The reagent channel has a spotting zone holding dried reagents and a high hydraulic resistance. The three channels merge at the end of the RI at a capillary valve that prevents the formation of air bubbles. RIs occupy a marginal area on the chips (typically less than 1 mm²) and are simple to fill with reagents using an inkjet spotter. During filling, reagents reconstitute in the reagent channel and exit the RI with a dilution factor that corresponds to the relative hydraulic resistance of the channels forming the RI. We exemplify the working principle of RIs by (i) distributing ~ 100 pg of horseradish peroxidase (HRP) in different volume fractions of a 1 μ L solution containing a fluorogenic substrate for HRP and (ii) performing an immunoassay for C-

reactive protein (CRP) using 450 pg of fluorescently-labeled detection antibodies (dAbs) that reconstitute in 5 to 30% of a 1 μ L sample of human serum. RIs preserve the simplicity of lateral flow assays while providing a great degree of control over the integration and release of reagents in a stream of sample. We believe this concept to be broadly applicable to microfluidic devices as used for biological assays in general. This chapter is based on Microfluidic device with auxiliary and bypass channels, L. Gervais, E. Delamarche, M. Hitzbleck, CH9-2010-0080 and on Controlled release of reagents into capillary-driven microfluidics using reagent integrators, M. Hitzbleck, L. Gervais, E. Delamarche, Lab on a Chip, 2011.

4.2 Introduction

Biological assays in general and immunoassays in particular rely on the interaction between analytes and a number of reagents to generate a measurable signal, the concentration of which is related to the amount of analyte in the sample. In the case of assays done in clinical laboratories or in research environments, reagents are typically performed sequentially using automated instruments or by hands with multiple pipetting steps and using cartridges containing reagents, large amounts of stock solution or components from kits.

The miniaturization and integration of tests on microfluidic devices provides opportunities for performing multiplexed tests, rapidly, using small volumes of samples and reagents, and in the field or point-of-care settings. However, implementing assays on microfluidic platforms also brings the challenge of integrating and releasing reagents during operation of the device. With DNA-based assays, reagents that might need to be integrated in a device can be chemicals for cell lysis, enzymes for PCR, nucleotides, oligonucleotide primers and probes, and substrates and cofactors for enzymes. [342] Similarly, reagents for immunoassays can be cAbs, dAbs carrying a signal-generating center (a fluorophore, an electroactive center or an enzyme with substrates), blocking reagents for minimizing non-specific signal, salts, acids/bases for pH adjustment, and compounds (e.g. carbohydrates) to stabilize and preserve proteins in the dry state.

Numerous proof-of-concepts exist for releasing reagents that are integrated in microfluidic devices upon actuation. [343, 344] A striking example is the integration of

an array of droplet generators and dispensers inside a microfluidic chip that can release 20 pL droplets of reagents on millisecond timescale. [345] Taking recent examples of centrifugal microfluidics (also referred to as “lab on a CD”), Ko et al. used a laser to melt a wax containing iron oxide nanoparticles to mix preloaded reagents in a blood sample for detecting antigens related to Hepatitis B virus. [110] Lutz et al. implemented an isothermal amplification and detection of DNA analytes using preloaded solutions and lyophilized reagents that mixed upon opening a valve using acceleration and pneumatic pressure. [44] Focke et al. used siphon valves and centrifugation to mix a sample with reagents for primary and secondary PCR-based amplification of a specific sequence of MRSA. [346] Abi-Samra et al. used series of wax valves having different melting temperatures for storing and releasing liquid reagents on CD-microfluidic platform. [347] Additional demonstrations of releasing chemicals upon actuation have been worked out in the context of drug release. These include the well-known example of controlled release of chemicals from Si microcontainers following the electrodisolution of a thin gold membrane [348] and methods based on nanoparticles that, upon heating, release chemicals due to collapse [349] or increased permeability. [350]

Actuation-based methods for releasing chemicals in microfluidics and elsewhere are flexible and can be extremely precise but necessitate either peripherals or costly (and sometimes complex) manufacture of chips. In comparison, the simplicity of manufacture and use of passive microfluidics and lateral flow assays make these tests attractive for broad use for *in vitro* diagnostics. Lateral flow assays, as illustrated in Figure 4.1, are still used 30 years after their inception and provides a benchmark for developing novel tests on a microfluidic format. In a lateral flow assay, and more specifically in an immunoassay, a sample is added at the beginning of a strip and migrates over an area containing dAbs in a dry state. The dAbs reconstitute in the filling front of the sample and bind analytes. The analyte-dAb complexes are captured downstream by surface-immobilized cAbs. The rest of the sample (not containing or containing less dAbs) rinses unbound dAbs away from the capture area. An optical or fluorescent label on the dAb contributes a signal that is proportional to the concentration of analyte in the sample. So far, most efforts undertaken to improve lateral flow assays focused on (i) transposing the concept of lateral flow assays

to microfabricated or micromolded microfluidics for improved flow control and accuracy of the tests, [31,98] (ii) varying the materials used for the flow paths for multiplexed analysis and/or achieving 3D flow paths, [53,351] (iii) substituting antibodies with various types of reagents, and (iv) improving signal generating species. Surprisingly, the integration and controlled release of reagents in entirely passive microfluidics for immunoassays, to the best of our knowledge, has not been specifically addressed. Combining active flow in microfluidics with passive release of reagents from cavities or porous pads is a good starting point to study release profiles and stability of proteins for microfluidic-based immunoassays. [336,352,353]

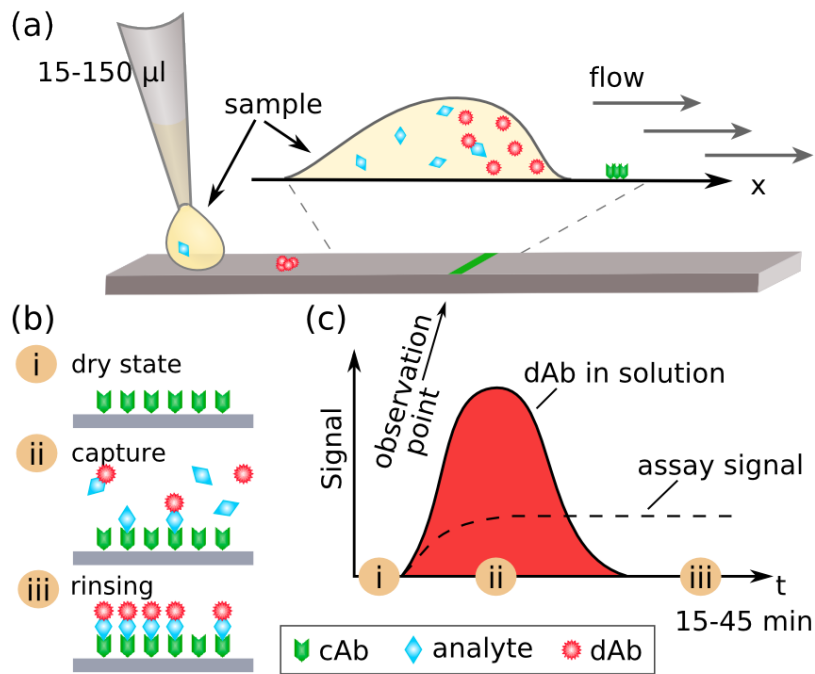


Figure 4.1: Illustration of a typical lateral flow immunoassay. (a) Sample is introduced onto a test strip having a line of cAb receptors and deposited dAb reagent. Capillary forces draw the sample through the test strip and dAbs reconstitute in the filling front of the sample. (b) Snapshots of the capture area at different time periods during the assay ((i) dry, (ii) during capture of analyte and (iii) after rinsing) and (c) corresponding assay signal evolution on the capture area. Here, both dAbs in solution (solid line) and immobilized on the capture area (dashed line) contribute to the fluorescence signal.

While some of the above-mentioned examples succeeded in integrating reagents into microfluidics for effecting immunoassays, many of these examples still require some

handling from an operator or actuation. In the case of lateral flow assays, the integration of reagents into pads serves the purpose of this technology, i.e. essentially a low cost of manufacture of the devices. But this method is essentially driven by prior experience and follows trial and error methods when it needs to be adjusted for specific assay conditions. Here, we describe RIs, which are functional microfluidic elements inside which small amounts of reagents can be incorporated and released with a controlled profile into a stream of sample.

4.3 Experimental

4.3.1 Chemicals, biochemicals and PDMS

Deionized water with a resistivity $\sim 18.2 \Omega \text{ cm}^{-1}$ was prepared using a Simplicity 185 system (Millipore, Billerica, MA). PBS was prepared by dissolving tablets (Dulbecco's, Sigma-Aldrich, St. Luis, MO, USA) in deionized water. Lyophilized BSA (Sigma-Aldrich) was reconstituted 1 % w/v in PBS. Ethanol (puriss > 99.8 %, Sigma-Aldrich) was used for cleaning Si chips and PDMS. PDMS was prepared by mixing Sylgard 184 pre-polymers (Dow Corning, Midland, MI, USA) with curing agent at a ratio of 10:1 using a DOPAG mixer (Cham, Switzerland). The PDMS mixture was cured in polystyrene Petri dishes at 60°C for at least 48 h. The resulting 1-mm-thick PDMS layers were cut into the size of Si chips. For immunoassays, cAbs were patterned on PDMS using Si stencils as reported elsewhere. [222] PDMS surfaces were passivated by immersion into a BSA solution for 30 min, rinsed with PBS and deionized water and dried using N₂. The buffer for enzymatic reactions had 0.1 M Trizma[®] (>99%, reagent grade, Sigma-Aldrich) and 10 mM MgCl₂ (Sigma-Aldrich). Amplex[®] Red reagent (Invitrogen, Carlsbad, CA, USA) was dissolved at a concentration of 10 mM in DMSO and stored in argon atmosphere at -80°C, protected from light. Prior to use, Amplex Red[®] was diluted to 0.1 mM in the reaction buffer. An aqueous solution of 20 mM H₂O₂ was mixed with the reaction buffer to obtain a final concentration of 10 μ M. The H₂O₂ solution was used for experiments within less than 4 h. HRP was obtained in lyophilized form from Sigma-Aldrich.

Samples for immunoassays were prepared by spiking human, CRP-free serum (HyTest,

Turku, Finland) with human CRP (HyTest). Anti-CRP-C2 and Anti-CRP-C6 Abs were purchased from Hytest and were used as cAb and dAb, respectively. Patterning of cAbs was realized with a solution of $125 \mu\text{g mL}^{-1}$ anti-CRP-C2 in PBS. Detection Abs were labeled with Alexa Fluor 647 following the instructions of the corresponding labeling kit (Invitrogen). The number of fluorophores per protein was approximately four, as determined by an Eppendorf BioPhotometer (Hamburg, Germany).

A solution of 2% v/v Hellmanex II (Hellma, Mullheim, Germany) was used to clean the inkjet glass pipettes. Reagents for inkjet deposition were composed of 34 mg mL^{-1} D-(+)-trehalose (Sigma-Aldrich) and $350 \mu\text{g mL}^{-1}$ L-phenylalanine (Sigma-Aldrich) mixed with $250 \mu\text{g mL}^{-1}$ dAb or HRP for immunoassays and enzymatic reactions, respectively. Si chips were treated with 3-methacryloxypropyltrichlorosilane (95%, ABCR, Karlsruhe, Germany) in the gase phase and 3-(2-aminoethylamino)propyltrimethoxysilane (Sigma-Aldrich) 1% v/v in deionized water.

4.3.2 Fabrication of chips and preparation

Microfluidic chips were fabricated in Si using a two-layer-photolithography process. A $0.5\text{-}\mu\text{m}$ -thick SiO_2 layer was grown on a 4 inch, single-side polished Si wafer (Siltronix, Geneva, Switzerland). An adhesion promoter (hexamethyldisilazane) and an approximately $1.6\text{-}\mu\text{m}$ -thick layer of photoresist (AZ 6612, Microchemicals, Ulm, Germany) were spin coated one after another onto the wafer and soft baked for 1 min at 110°C . For the first layer of microfluidic structures, which had all structures, the resist was exposed through a quartz/Cr mask (Photronix, Dresden, Germany) to 6.5 mW cm^{-2} ultraviolet light for 15 s using a mask aligner (MA/BA6, Süss Microtech, Garching, Germany) and developed. The structures were etched into the Si layer using buffered hydrofluoric acid (7:1 volume ratio of 87.5% NH_4F in water to 12.5% HF in water; danger). After stripping the first layer of photoresist, a second layer of photoresist ($\sim 7 \mu\text{m}$ thick) was spin coated and soft baked. The second mask was used to pattern all microfluidic structures but the reaction chamber in the photoresist. Exposure time in the mask aligner was 45 s and was followed by development of the resist. Deep reactive ion etching (AMS-200SE, Alcatel Micro Machining Systems) was used to etch the channel structures into the Si chip.

After the first layer was etched 20 μm deep into the wafer, the resist was stripped and the image of the second mask was transferred over a depth of 40 μm using the remaining SiO_2 mask. The final chip had 60- μm -deep structures and 20- μm -deep reaction chambers.

Two treatments based on silanes were used to define the wetting properties of the chips. First, Si chips were covered with Nitto Tape (Powatec, Cham, Switzerland) except for the region around the delay valve ending the RI. Gas phase deposition of methacryloxypropyltrichlorosilane was done in a desiccator at 150 mbar for 25 min. The resulting advancing contact angle for water of the chip surface was 53° . Then, the tape was removed and chips were immersed into a solution of 3-(2-aminoethylamino)propyltrimethoxysilane for 30 min, rinsed with deionized water and dried using N_2 to yield an advancing contact angle of 45° . Chips were used within a day following this treatment.

4.3.3 Patterning of cAbs

Capture Abs (anti-CRP-C2) were patterned on PDMS using Si stencils. The stencils were cleaned in an air-based plasma at 200 W and 0.7 Torr base pressure for 2 min. On the surface of the PDMS sheet, which previously faced the Petri dish, the polished side of the Si stencil was placed. A solution of cAbs was pipetted in the trenches of the stencils and left for 15 min at room temperature in a humid environment. Stencils were rinsed with PBS, deionized water and placed in a Petri dish containing a solution of BSA before separating them from the PDMS. After 15 min, the PDMS was rinsed with PBS, deionized water, and dried using N_2 . Stencils were cleaned with EtOH and deionized water for re-use.

4.3.4 Enzymatic Reaction

HRP was spotted onto the chip using an Autodrop MD-P-705-L (Microdrop, Norderstedt, Germany) inkjet dispenser. A piezo-driven pipette (AD-K-501, Microdrop) with a nozzle opening of 70 μm was positioned in close proximity over the spotting zone. Typically, five droplets of HRP solution, each about 180 pL in volume, were spotted. Spotting at low frequency (~ 0.5 Hz) ensured that spreading of the HRP solution out of the spotting zone was limited by the rapid evaporation of the droplets. Chips were stored dry and protected

from light and were used within 4h. The sample with substrate for the enzymatic reaction was prepared by mixing Amplex red[®] with H₂O₂ in the reaction buffer at a ratio of 1:10 mM. The chip was covered with a PDMS layer and 15 μ L of sample was pipetted onto the loading pad. Before filling, during and after rinsing of the RI, images of the RI zone were acquired through the PDMS in bright field and fluorescence mode. When capillary pumps of the chip were completely filled, fluorescent images of the pumps were taken. After use, chips were cleaned with water and ethanol. Remaining organic contaminations were oxidized in an UV-ozone Photoreactor (PR-100, Ultra-Violet Products, Upland, CA, USA).

4.3.5 Immunoassay

Similar to the experiments described above, chips were prepared by dispensing 20 drops of dAb solutions into RI spotting zones. Samples for testing RIs were human serum spiked with 10 ng mL⁻¹ of CRP. The PDMS with lines of cAbs was placed so as to have the cAb lines crossing the reaction chambers of the chip. A volume of 15 μ L of sample was pipetted in the loading pad and fluorescence images of the cross-sectional area of reaction chambers and cAb lines were acquired at regular intervals until the capillary pumps were filled.

4.3.6 Instrumental setup and data acquisition

A fluorescence microscope (Eclipse 90i, Nikon, Japan) was used to acquire optical and fluorescence images. Fluorophores were excited using a 100 W Hg arc lamp and micrographs were captured using a Peltier-cooled, black and white CCD camera (DS-1QM, Nikon) and filter sets for resorufin and Alexa Fluor 647. To visualize the resorufin distribution in the capillary pumps, 2 \times 7 fluorescent images at 4 \times magnification were taken and digitally stitched. Images at 10 \times magnification were taken with increasing time intervals of 15 s – 1 min for 30 min. In order to minimize bleaching effects, the shutter was opened only during the capture. Quantitative data evaluation was done by NIS-Elements software (Nikon) and Matlab (MathWorks, Natick, MA, USA) and during immunoassays, image acquisition was automated using a macro function implemented on NIS-Elements.

4.4 Results and discussion

The goal of using RIs is to provide a zone inside which reagents are easily deposited using an inkjet and reconstitute in a defined filling front of sample. The key concept of RIs is to distribute an incoming stream of sample to a channel containing reagents (the “reagent channel”) and parallel channels (called “diluter channels”). The relative hydraulic resistance of the channels defines the dissolution profile of reagents after the RI and before passing over capture areas. Figure 4.2 shows an example of a RI. The RI starts with a junction that splits an incoming sample into three parallel channels (Fig. 4.2a). The reagent channel starts with a 10- μm -wide microchannel, which generates a high capillary pressure and high flow resistance, and continues with the spotting zone. The spotting zone here is up to 100 μm in width and has a volume of 2.5 nL. It can be targeted by an inkjet for the deposition of dAbs into the chip. Alignment requirements for the inkjet nozzle with the spotting zone are relaxed due to the extended width of the structure. The diluter channels merge with the reagent channel at the end of the RI in an area that has a capillary delay valve. [159] When the chip is filled, the dAbs present in the spotting zone reconstitute in the stream of sample flowing in the reagent channel and the valve at the end of the RI ensures that the three filling fronts merge before proceeding to the outlet. This prevents the formation of air bubbles that may otherwise block flow through one of the channels. The aspect ratio and dimensions of the structures forming the RI shown here were selected to allow for their production in polymers by means of mold injection if needed. [40]

The RI is fully compatible with the delivery of reagents using inkjet technology, which is beneficial for economizing reagents and preparing chips at high throughput (Fig. 4.2b). The alignment of the inkjet nozzle over the spotting zone of the RI takes a few minutes when done manually and the deposition itself is completed within seconds. Typically, 5 to 20 drops each 180 pL in volume are spotted. The drops are ~ 70 μm in diameter and easily fit into the spotting area. The narrow structures with high capillary pressure in the reagent channel help placing the liquid reproducibly in the channel and take care of small positioning variations of droplets in the structure. To limit spreading of reagent throughout the channels, especially to prevent diluter channels from being contaminated by reagents, spotting frequencies of 0.5-1 Hz were used to accumulate droplets. If a large volume of

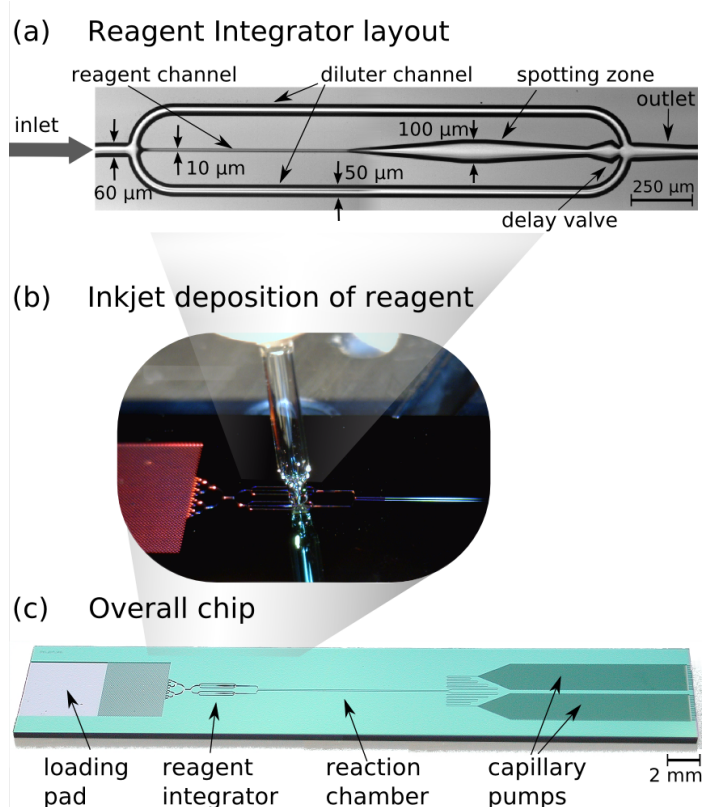


Figure 4.2: Concept of the microfluidic RI and implementation into a capillary-driven microfluidic chip. (a) Optical micrograph of a RI structure in Si with typical dimensions. (b) Image showing the delivery of a solution of reagent (e.g. dAb) into the spotting zone of a RI using an inkjet printer. (c) Image of a Si chip comprising various microfluidic elements such as the RI.

reagent solution or faster deposition is needed, spotting droplets at a lower frequency and/or heating the chip during spotting can be done to gradually evaporate the solution while accumulating droplets.

Figure 4.2c shows a microfluidic chip having one loading pad and two sets of RIs, reaction chambers and capillary pumps. The PDMS layer having patterned lines of capture antibodies and used to seal the chip is omitted in this image. To perform an immunoassay, a user only needs to pipette $\sim 15 \mu\text{L}$ of sample into the loading pad. The sample is drawn from there by capillary action up to the capillary pumps. The footprint of each RI in this chip is very small ($0.4 \times 2.3 \text{ mm}^2$) and does not impact the overall dimensions of the chip.

The functioning of a RI is shown in Figure 4.3a by integrating HRP to the RI, releasing the enzyme, and using it to convert a fluorogenic substrate present in a sample into a

fluorescent product. The optical micrograph of the RI after drying the HRP solution in the RI shows the confinement of the enzyme reagent to the spotting zone. Following the addition of 15 μL of sample containing Amplex red[®], HRP dissolves, catalyzes the production of resorufin, and is gradually transported outside the RI.

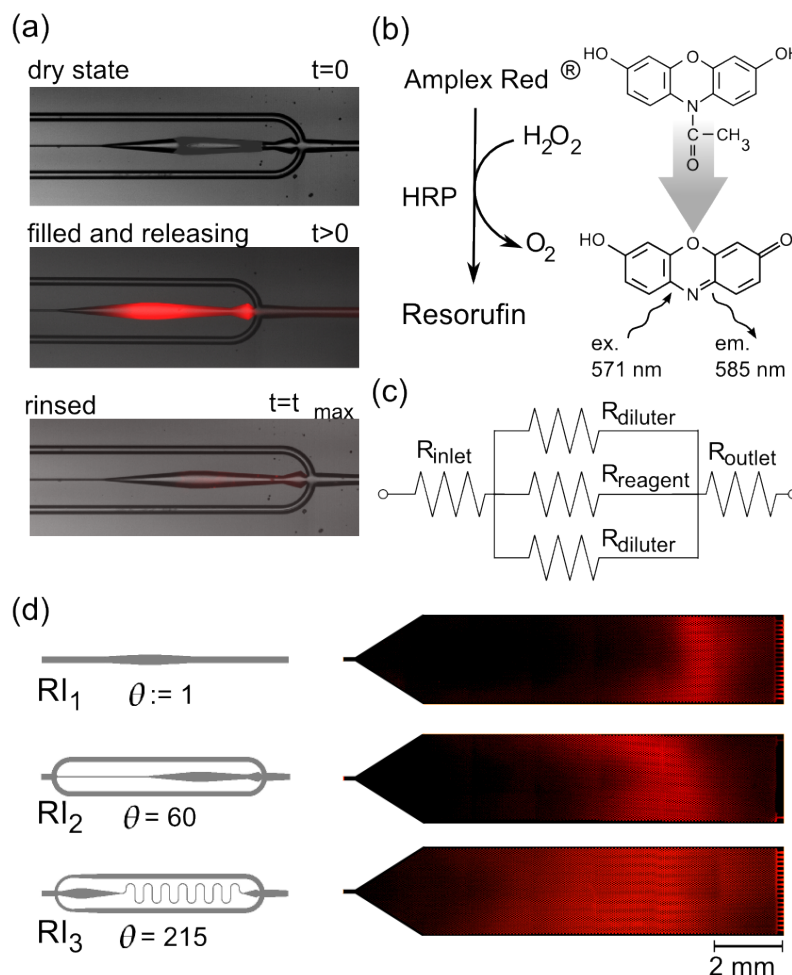


Figure 4.3: Example of RIs having various dilution factors and their use for the integration and release of enzymes in a defined volume fraction of sample. (a) Combined optical and fluorescent images of a RI having HRP in dry state, during the release of HRP into a solution containing Amplex Red[®] and H₂O₂ as visualized by the fluorescent product resorufin, and when microfluidic pumps are filled and the RI is rinsed. (b) Schematic of enzymatic reaction between HRP and substrates. (c) Electrical equivalent circuit of the RI in (a). (d) Design of RIs having various dilution factors and corresponding fluorescence images of filled capillary pumps containing HRP (and resorufin).

The equivalent electric circuit in Fig. 4.3c represents the hydraulic resistances of the

flow path before and after the RI and of the channels composing the RI. The number and characteristic dimensions of the diluter channels can easily be varied. We arbitrarily define the ratio between the resistance of the reagent channel and the sum of the resistances composing the RI as the dilution factor Θ . This factor provides a simple design criterion for ranking RIs based on their dilution power. Examples of RIs having very low ($\Theta = 1$), medium ($\Theta = 60$) and high ($\Theta = 215$) values are presented in Figure 4.3d. In these examples, the simplest RI corresponds to a spotting zone without diluter channels whereas the RI having the highest dilution factor has two 50- μm -wide diluter channels and a 10- μm -wide, meandering reagent channel. The distribution of resorufin in the filled capillary pumps show the effect of these RIs on the dissolution profiles of HRP. A small dilution factor results in a more concentrated filling front of HRP in the sample and therefore a stronger confinement of resorufin toward the end of the pump. It should be noted that the distribution of HRP is only indirectly revealed by the spread of the fluorescence signal in the pumps because the microstructures in the pumps that are used to encode capillary action also act as mixers to some extent.

The controlled release profiles of dAbs during immunoassays detecting CRP in human serum are shown in Figure 4.4. As a first step, the quantity of dAbs passing in the reaction chamber of a chip was calibrated by measuring the fluorescence intensity of solutions of dAb having known concentrations. Then, dAbs were integrated into RIs similar to the ones presented above and the dissolution of the dAbs was first monitored in the reaction chamber at areas between lines of cAbs. The rates of dissolution of dAbs are strikingly different: 75% of the dAbs released and passed the reaction chamber within only ~ 1 min when the RI was a simple spotting zone. While this release might be suited for fast immunoassays for which the concentration range of analytes is high, such a fast release is not desirable for high-sensitivity assays. [354] Release of dAbs from RIs having a dilution factor of 60 and 215 leads to having dAbs distributed in a much larger volume fraction of the sample. As a consequence, it takes ~ 3 min ($\Theta = 60$) and ~ 6 min ($\Theta = 215$) for 75% of the spotted dAbs to pass through the reaction chamber, in which case the incubation time of the assay is extended.

In practical terms, an assay does not follow the dissolution profile of dAbs in solution

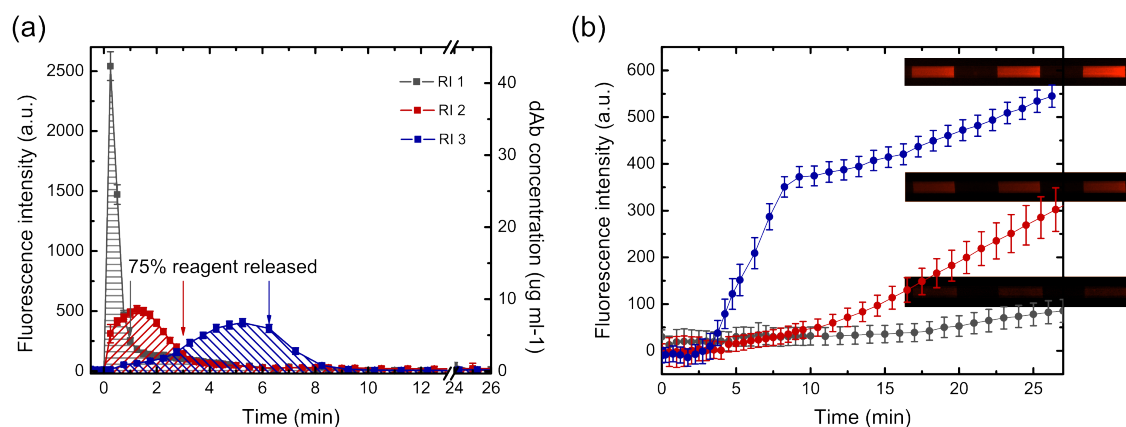


Figure 4.4: Release of dAbs from various RIs and their contribution to signal generation over capture areas during a one-step immunoassay detecting CRP at a 10 ng mL^{-1} concentration in human serum. (a) Graphs (square) showing the release of dAbs in $1 \mu\text{L}$ of serum as revealed by the fluorescence signal and corresponding concentration of dAbs measured in between capture areas. (b) Temporal evolution of the specific fluorescence signals on the capture areas (circle) for the corresponding RIs indicated in (a). The insets are fluorescence micrographs obtained on the capture areas when capillary pumps are filled with the sample.

but measures directly the fluorescence corresponding to the specific capture of analyte-dAb complexes on cAb areas, as shown in Fig. 4.4b. The evolution of the fluorescence signals reflects how RIs having larger dilution factors contribute to longer incubation times of the capture areas with analyte-dAb and therefore to higher signal for equivalent CRP analyte concentrations. The best signal here is obtained using the RI having a large dilution factor (RI_3 , $\Theta = 215$) in which case most of the signal is obtained after 9 min. In comparison, the fastest RI (RI_1 , $\Theta = 1$) released a “spike” of dAbs that was inefficient to form analyte-dAbs complexes in a sufficiently large volume fraction of the sample for optimal capture conditions.

4.5 Conclusions

The integration of reagents and specifically their release in microfluidics has a profound influence on the performance of a microfluidic device. We believe that this topic has been largely omitted in the development of microfluidic devices and suggest that RIs that can be conceptualized with simple design rules and easily implemented into microfluidic chips

can remedy this situation. There are many intriguing possibilities to implement RIs into microfluidics that go well beyond the work presented here. For example, a parallel array of RIs might be utilized to integrate various reagents and release them all with a specific profile. By merging the outlet of a RI with a series of subdividing diluter channels, an ensemble of streams with a discontinuous concentration gradient of a sample might be produced. The concept of a RI is compatible with microfluidics using active pumping and may help when different chemicals need to be released in a stream of liquid with low dead volume.

5

Conclusion and Outlook

Capillary-driven microfluidic chips were fabricated and demonstrated as viable next generation point-of-care diagnostics devices. These microfluidic chips, in addition to detecting proteins, can also be used to detect DNA analytes for molecular diagnostics. A variety of surface chemistries can be used to attach biological molecules to surfaces. The avidin-biotin interaction is the strongest known non-covalent biological binding. Immobilized avidin has been used to attach a variety of biotinylated protein and nucleic acid receptor probes to the surfaces of biosensors. [355, 356] Additionally, the immobilization of biotinylated DNA onto chemically treated and avidin coated microscope slides is a common technique in biochemistry. [357, 358] A similar molecular assembly was used to bind single stranded DNA (ssDNA) probes onto PDMS substrates and perform assays for the detection of nucleic acids. The concept of multistep capillary-driven microfluidic chips for the detection of short (~ 22 base pairs) nucleic acid fragments extracted from pathogenic DNA is shown in Figure 5.1.

We developed a protocol for immobilizing DNA receptors on a PDMS surface. Avidin was patterned onto PDMS as previously explained, [222] followed by the patterning of biotinylated DNA strands. We used these receptors to bind fluorescently-marked DNA analytes from 3 μL of solution using a capillary-driven microfluidic chip and rinsed with 1.5

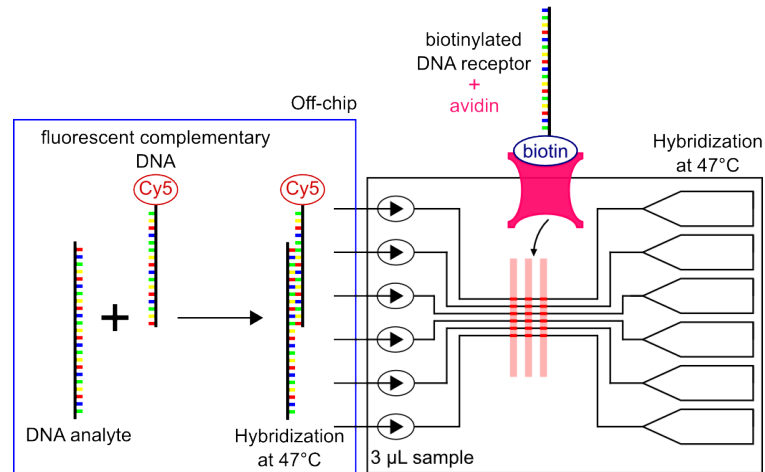


Figure 5.1: Concept for the detection of pathogen DNA on a capillary-drive microfluidic chip. The DNA from pathogens is extracted from a patient sample. The DNA is cleaved into short nucleic acid fragments that can be used to identify the pathogen. The sDNA analyte is incubated with fluorescent complimentary DNA at 47°C. The conjugate is pipetted onto a capillary driven microfluidic chip heated at 47°C. The complex of DNA analyte and fluorescent DNA binds to complimentary DNA immobilized via biotin-avidin interaction in the reaction chamber.

μL of pure buffer. DNA was detected in 3 min, with a sensitivity better than 10 nM, Figure 5.2. This is the first demonstration of DNA tests using our capillary-driven microfluidic chips.

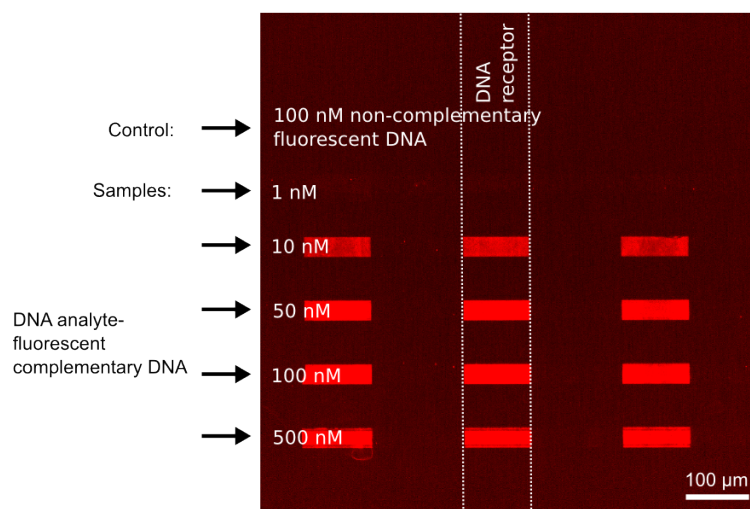


Figure 5.2: Fluorescence micrograph of the multistep detection of pathogenic DNA on a capillary driven microfluidic chip. The chip was kept at 47°C. A 3 μL sample containing sDNA strands at concentrations ranging from 1 nM to 500 nM was pipetted onto a chip with 6 independent loading pads and microfluidic flow paths. A complementary fluorescently labelled sDNA strand was pipetted onto each loading pads, followed by 5 μL of rinsing buffer. The detection regions show the complex of pathogenic sDNA, fluorescent-sDNA, and Avidin-sDNA immobilized on the surface in 6 independent reaction chambers.

The next step was to simplify the test by removing the need for a rinsing step. A one-step capillary-driven microfluidic chip for the detection of pathogenic DNA is shown in Figure 5.3. Fluorescent DNA detection strands are spotted in the microfluidic flow path as explained previously explained. [31] The microfluidic chips detect DNA analyte spiked in 5 μL of buffer within 10 min and at 55°.

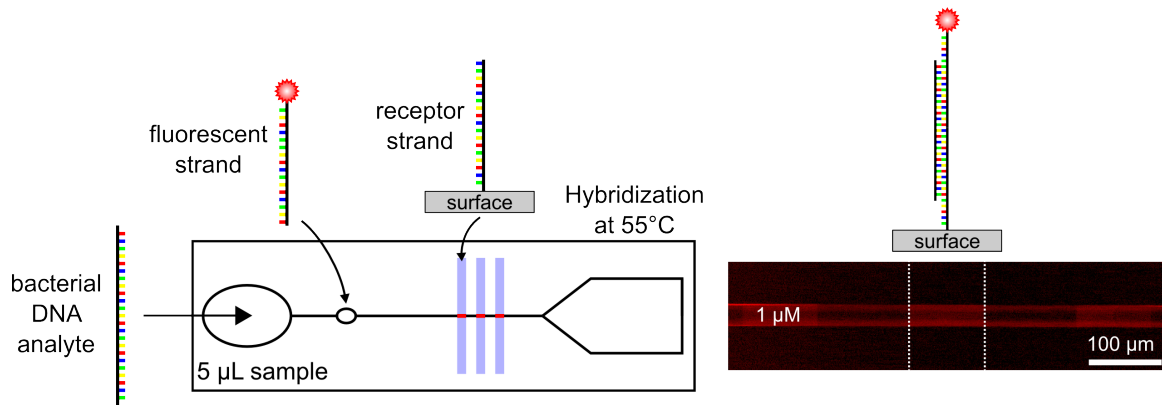


Figure 5.3: One-step detection of pathogenic DNA on a capillary-driven microfluidic chip. The scheme is shown on the left. A 5 μL sample containing pathogenic sDNA prepared outside of the chip is pipetted onto the loading pad. The sample redissolves previously spotted fluorescently labelled complementary sDNA. The pathogenic sDNA hybridizes with the fluorescent-sDNA on the chip kept at 55°C. In the reaction chamber the complex hybridizes with immobilised Avidin-sDNA. A fluorescence micrograph of the detection of 1 μM pathogenic sDNA is shown on the right.

We believe that the combination of microfluidic elements and integrated reagents into one-step capillary-driven microfluidic chips can be used to produce decentralized medical diagnostics devices. This devices when fabricated into plastics and used with low cost fluorescence detection readers can begin to make a direct impact by improving patients healthcare.

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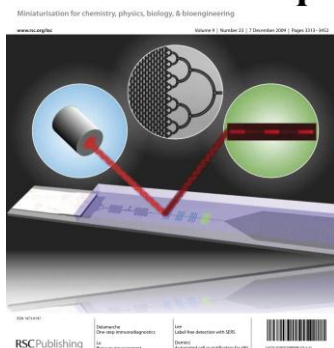
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- [358] B. Kaboord and R. Liermann, "Biotinylated DNA binding pierce avidin supports," Tech. Rep. Application Note # 14, Thermo Scientific - Pierce Protein Research, Apr. 2006.

LIST OF PUBLICATIONS – related to this thesis

Refereed Journal Publications

- [4] M. Hitzbleck, **L. Gervais**, E. Delamarche, *Controlled release of reagents into capillary-driven microfluidics using reagent integrators*, Lab on a Chip, 2011 (submitted)
- [3] **L. Gervais**, M. Hitzbleck, E. Delamarche, *Capillary-driven multiparametric microfluidic chips for one-step immunoassays*, Biosensors and Bioelectronics, 2011 (submitted)
- [2] **L. Gervais**, Nico de Rooij, E. Delamarche, *Microfluidic chips for point-of-care immunodiagnostics*, Progress Report, Advanced Materials, 2011
- [1] **L. Gervais** and E. Delamarche, *Toward one-step point-of-care immunodiagnostics using capillary-driven microfluidics and PDMS substrates*, Lab on a Chip (**cover article**), 9, 3313-3452, 2009

Lab on a Chip



Significant worldwide press coverage: 100+ articles on news sites, magazines, newspapers, TV and radio including BBC, EE Times, CNN, MIT Technology Review, 5200+ views on Youtube.

Patent:

- [1] CH9-2010-0080: **Luc Gervais**, Emmanuel Delamarche, Martina Hitzbleck, *Microfluidic device with auxiliary and bypass channels*

Proceeding Publications

- [3] **L. Gervais**, E. Delamarche, *Extended dynamic range capillary-driven microfluidics*, MicroTAS, Groningen, Netherlands, October 3-7, 2010
- [2] **L. Gervais**, E. Delamarche, *Point-of-care diagnostics with integrated reagents based on capillary-driven microfluidics*, MicroTAS, Jeju, Korea, November 1-5, 2009
- [1] **L. Gervais**, M. Zimmermann, P. Hunziker, E. Delamarche, *One-Step Immunoassay on Capillary Driven Microfluidics*, MicroTAS, San-Diego, California, USA, October 12-16, 2008

Conference Oral Presentations

- [2] **L. Gervais**, *Microfluidics: from the bench to the bedside*, Molecular Diagnostics Europe, **Invited talk**, Cambridge Healthtech Institute, Hannover, Germany, October 6-7, 2010
- [1] **L. Gervais**, M. Zimmermann, P. Hunziker, E. Delamarche, *One-Step Immunoassay on Capillary Driven Microfluidics*, MicroTAS, San-Diego, California, USA, October 12-16, 2008

Conference Poster Presentations

[2] **L. Gervais**, E. Delamarche, *Extended dynamic range capillary-driven microfluidics*, MicroTAS, Groningen, Netherlands, October 3-7, 2010

[1] **L. Gervais**, E. Delamarche, *Point-of-care diagnostics with integrated reagents based on capillary-driven microfluidics*, MicroTAS, Jeju, Korea, November 1-5, 2009

LIST OF PUBLICATIONS – not related to this thesis

Refereed Journal Publications

[2] **L. Gervais**, M. Gel, B. Allain, M. Tolba, L. Brovko, M. Zourob, R. Mandeville, M. Griffiths and S. Evoy, *Immobilization of Biotinylated Bacteriophages on Biosensor Surfaces*, Sensors and Actuators: B-Chemical, 125, 615-621, 2007

[1] N. Nelson-Fitzpatrick, C. Ophus, E. Lubner, **L. Gervais**, Z. Lee, V. Radmilovic, D. Mitlin and S. Evoy, *Synthesis and characterization of Au-Ta nanocomposites for nanomechanical cantilever devices*, Nanotechnology, 18, 355303, 2007

Proceeding Publications

[1] **L. Gervais**, M. Gel, S. Evoy, L. Brovko, M. Griffiths, B. Allain, R. Mandeville, *Surface Functionalization Strategy for Highly Selective Phage-based Bacterial Detection*, Foundations of Nanoscience: Self-Assembled Architectures and Devices, Snowbird, Utah, USA, April 23-27, 240-244, 2006

Conference Oral Presentations

[5] **L. Gervais**, S. Evoy, L. Brovko, M. Griffiths, B. Allain, R. Mandeville, *T4 Bacteriophage functionalized cantilevers for highly specific bacterial detection*, International Workshop on Nanomechanical Sensors, Montréal, Québec, Canada, May 27-31, 2007

[4] **L. Gervais**, S. Evoy, L. Brovko, M. Griffiths, B. Allain, R. Mandeville, *T4 Bacteriophage functionalized cantilevers for highly specific bacterial detection*, iCORE Alberta ECE Graduate Research Symposium, Edmonton, Alberta, Canada, May 4, 2007

[3] **L. Gervais**, S. Evoy, L. Brovko, M. Griffiths, B. Allain, R. Mandeville, *Microcontact Patterning of T4 Bacteriophages for Highly Specific Bacterial Detection*, Material Research Society (MRS) Spring Meeting, San-Francisco, California, USA, April 10-17, 2007

[2] C. Ophus, R. Mohammadi, E. Lubner, **L. Gervais**, B. Olsen, C. Harrower, C. Gilkison, N. Nelson-Fitzpatrick, L. M. Fischer, K. Westra, Z. Lee, V. Radmilovic, U. Dahmen, S. Evoy, D. Mitlin, *Metal Nanocomposites for NEMS and AFM Applications*, 2006 American Vacuum Society (AVS) Fall Meeting, San-Francisco, California, USA, November 12-17, 2006

[1] C. Ophus, R. Mohammadi, E. Lubner, **L. Gervais**, N. Nelson-Fitzpatrick, L. M. Fischer, A. Murray, Z. Lee, V. Radmilovic, U. Dahmen, S. Evoy, D. Mitlin, *Nano-Composite Metal Thin Films for MEMS, NEMS and Energy Applications*, 2006 Material Research Society (MRS) Spring Meeting, San-Francisco, California, USA, April 17-21, 2006

Conference Poster Presentations

[4] N. R. Glass, A. Singh, **L. Gervais**, M. Gel, B. Allain, M. Tolba, L. Brovko, R. Mandeville, M. Griffiths and S. Evoy, *Bacteria Detection on Bacteriophage Functionalized Surfaces and Cantilevers*, Material Research Society (MRS) Spring Meeting, San-Francisco, California, USA, March 24-28, 2008

[3] N. Nelson-Fitzpatrick, **L. Gervais**, C. Ophus, E. Lubber, Z. Lee, V. Radmilovic, U. Dahmen, D. Mitlin and S. Evoy, *Gold-Tantalum Nanocomposite as Structural Material for Resonant NEMS Biosensing Cantilevers*, Material Research Society (MRS) Spring Meeting, San-Francisco, California, USA, April 10-17, 2007

[2] **L. Gervais**, M. Gel, S. Evoy, L. Brovko, M. Griffiths, B. Allain, R. Mandeville, *Surface Functionalization Strategy for Highly Selective Phage-based Bacterial Detection*, Foundations of Nanoscience: Self-Assembled Architectures and Devices, Snowbird, Utah, USA, April 23-27, 2006

[1] **L. Gervais**, M. Gel, S. Evoy, L. Brovko, M. Griffiths, B. Allain, R. Mandeville, *Surface Functionalization Strategy for Highly Selective Phage-based Bacterial Detection*, NanoForum Canada: Nanoscience and Nanotechnology Forum, Edmonton, Alberta, Canada, June 20-22, 2006

Luc Gervais

EDUCATION

2009 - 2011	Swiss Federal Institute of Technology (EPFL) Doctor of Philosophy in Microsystems and Microelectronics <ul style="list-style-type: none">Conceived point-of-care diagnostics chips for the detection of proteins and DNA.	Switzerland
2005 - 2007	University of Alberta & National Institute for Nanotechnology Master of Science in Electrical and Computer Engineering <ul style="list-style-type: none">Conceived bacteria-detecting microchips, in collaboration with Biophage Pharma (startup).	Canada
2004 - 2005	National Taiwan University (國文臺灣大學) Chinese Language Certificate <ul style="list-style-type: none">Studied Chinese language and culture full-time. Complete immersion in a Mandarin Chinese environment.	Taiwan
2000 - 2004	Concordia University Bachelor of Computer Engineering Thesis: Design and implementation of an ARM vector floating point coprocessor	Canada

WORK EXPERIENCE

Jul. 2007 - Apr. 2011	IBM Research – Zurich PhD Student <ul style="list-style-type: none">Developed point-of-care diagnostics devices that had synergies with a major IBM healthcare initiative.Conducted interviews and presentations with international journalists and partners.Worked on a project for the diagnosis of cardiac markers with Basel University Hospital, Bühlmann Labs.Worked on the development of microfluidics for the detection of DNA from pathogens with Coris BioConcept.	Switzerland
Oct. 2002 - Sept. 2003	Unlikely Games (startup) Game Developer <ul style="list-style-type: none">Programmed an original action role-playing game for the Sony Playstation 2 that was demonstrated at the Electronics Entertainment Exposition in Los-Angeles.	Canada
May - Aug. 2002	Boehringer Ingelheim Computational Chemistry Developer <ul style="list-style-type: none">Programmed software to simulate and visualize proteins that play an important role in antiviral drug development.	Canada

Sept. - Dec. 2001	Steltor (startup acquired by Oracle in 2002) Quality Assurance Specialist <ul style="list-style-type: none">Improved Corporate Time software features and fixed bugs.	Canada
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AWARDS AND RECOGNITION

2010	IBM research division accomplishment "Chips for Life"
2010	Microfluidic work featured during IBM's 2010 Annual Meeting of Stockholders
2009, 2010	Worldwide press coverage of one-step diagnostic chip: 100+ articles on news sites, magazines, newspapers, TV and radio including BBC, EE Times, CNN, Bloomberg, MIT Technology Review, 5200+ views on Youtube.
2009	Work on point-of-care devices presented to IBM's Board of Directors and IBM's CEO during their annual strategy meeting
2006, 2007	Queen Elizabeth II Master's Scholarship (9.3k\$ per annum)
2005	Taiwan National Science Council Graduate Scholarship (15k\$ per annum)

LANGUAGES

English: native
French: native
Chinese: competent
German: competent
Spanish: competent

EXTRA CURRICULAR ACTIVITIES

Swimming Instructor and Lifeguard: Red Cross Instructor & Examiner, National Lifeguard
Ski Instructor and Competitor: Level 1 (CSIA), Concordia University ski team
Dragon Boat: McGill/Concordia team, Montréal Dragon Boat Festival Competitions
Music: clarinetist in the Collège Notre-Dame Wind Band